2001

Analysis and Mathematical Modeling of Autonomously Oscillating Yeast Cultures.

Abdelqader M. Zamamiri
Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_disstheses/256

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
ANALYSIS AND MATHEMATICAL MODELING OF AUTONOMOUSLY OSCILLATING YEAST CULTURES

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemical Engineering

by

Abdelqader M. Zamamiri
B.S., University of Jordan, 1994
M.S., Louisiana State University, 1998
May 2001
Dedicated to

my lovely wife, Lina

and

my wonderful parents

for their endless love and continuous support.
Acknowledgments

Financial support from the National Science Foundation, grant BES-9522274, is gratefully acknowledged.

I owe my advisor Prof. Martin Hjortsø my sincere gratefulness for his true guidance and continuous assistance. I have learned a lot from him and was always inspired and impressed by his knowledge and school of thought. I would also like to thank my co-advisor and teacher Prof. Michael Henson for his critical observations and the very useful discussions we had over the years. In addition, I owe my colleagues Dr. Gülñur Birol, Guang-Yan Zhu, Yongchun Zhang and Prashant Mhaskar lots of thanks for their assistance and helpful remarks.

I want to thank my parents, my in-laws and my family for their unending faith in me and their continual support of my cause.

Last but not least, I owe the most gratitude for my wife, Lina, not only for her exceptional tolerance and patience, but also for her continuous encouragement and everlasting love.
# Table of Contents

**ACKNOWLEDGMENTS** ................................................................. iii

**LIST OF TABLES** ........................................................................ vi

**LIST OF FIGURES** ...................................................................... vii

**ABSTRACT** ................................................................................ xi

**CHAPTER**

1 *INTRODUCTION* ........................................................................ 1
   1.1 Literature Review ................................................................. 1
   1.1.1 Autonomously Oscillating Yeast Cultures ......................... 1
   1.1.2 Cell Cycle of *Saccharomyces cerevisiae* ......................... 4
   1.1.3 Metabolic Pathways in *Saccharomyces cerevisiae* ............. 8
   1.1.4 Conceptual and Mathematical Models ............................. 11
   1.2 Statement of the Problem .................................................... 24

2 *MULTIPLE STABLE STATES AND HYSTERESIS IN CONTINUOUS, OSCILLATING CULTURES OF BUDDING YEAST* .............. 31
   2.1 Introduction ........................................................................... 31
   2.2 Materials and Methods ......................................................... 34
   2.2.1 Preparation of Randomized-Cells Inoculum (RCI) .............. 37
   2.3 Results .................................................................................. 38
   2.3.1 Randomized Cell Inoculum ................................................. 38
   2.3.2 Switch Experiments ......................................................... 40
   2.3.3 Ramp Experiments ........................................................... 41
   2.4 Discussion ............................................................................. 44
   2.5 Conclusions .......................................................................... 50

3 *DYNAMICS ANALYSIS OF AN AGE DISTRIBUTION MODEL OF OSCILLATING YEAST CULTURES* ........................................... 51
   3.1 Introduction ........................................................................... 51
   3.2 Model Development and Method of Numerical Solution ........ 54
   3.2.1 Model Development ........................................................ 54
   3.2.2 Numerical Solution ......................................................... 61
   3.3 Results and Discussion ....................................................... 67
   3.3.1 Simulation Results .......................................................... 67
   3.3.2 Bifurcation Analysis Results ......................................... 75
   3.4 Conclusions .......................................................................... 82
4 Dynamics Analysis of a Hybrid Mass-Age Distribution Model of Oscillating Yeast Cultures .................................................. 83
4.1 Introduction ......................................................................................................... 83
4.2 Model Development ........................................................................................... 88
4.3 Numerical Solution ............................................................................................ 93
4.4 Model Simulations ............................................................................................. 95
  4.4.1 Analytical Jacobian Matrix .............................................................................. 100
  4.4.2 Steady State Analysis ......................................................................................... 111
4.5 Results and Discussion ..................................................................................... 119
4.6 Conclusions ....................................................................................................... 127

5 Summary ............................................................................................................. 130

Bibliography ........................................................................................................... 137

Appendix: Unpublished Experimental Results ..................................................... 142

Vita .......................................................................................................................... 156
List of Tables

1.1 Model equations for the special case shown in figure 1.4 .......................... 23
3.1 Parameters of the Age Distribution Model ............................................. 61
3.2 Parameters of the Transition and Division Intensity Functions ............... 61
4.1 Parameters of the Hybrid Mass-Age Distribution Model ..................... 100
List of Figures

1.1 A typical oscillation pattern of synchronous culture of *S. cerevisiae*. in terms of glucose concentration, exhaust CO$_2$%, ethanol concentration and percentage of dissolved oxygen (saturation at 100%) during two consecutive cycles. The operating conditions are: dilution rate, $D = 0.15$ hr$^{-1}$, glucose feed concentration, $S_f = 30$ g/L, and pH = 5.0.  3

1.2 Schematic diagram of *Saccharomyces cerevisiae* cell cycle. ............................... 6

1.3 Glucose consumption, ethanol production and consumption, percentage of dissolved oxygen (saturation at 100%) and the dry weight concentration during a batch culture of *S. cerevisiae* ..........................10

1.4 Trajectories in the time-age plane of perfectly synchronized subpopulations of mothers and daughters. ..............................21

2.1 Time course of exhaust CO$_2$ and ethanol concentration with addition of externally grown cells. An initially oscillating culture is switched to batch at 8.5 hr and back to continuous mode at 26 hr. At this time, indicated by the vertical arrow, a randomized cell inoculum is added. After a few oscillations non-oscillatory behavior was obtained under the same operating conditions where oscillatory behavior was observed previously. The non-oscillatory behavior was not due to imperfections in the feed as evidenced by the oscillations obtained after switching the system to the batch (105 hr) and then to the continuous mode again (123 hr) . . . .39

2.2 Progression of exhaust CO$_2$ and ethanol concentration for the experiments when the switch to the continuous operation took place at early lag. The vertical arrow indicates the time at which the culture was switched from batch to continuous mode. ................................. 41

2.3 Time course of the exhaust CO$_2$ concentration during two slow ramp changes in dilution rate. The culture is initially in a stable oscillatory mode at a dilution rate of 0.125 hr$^{-1}$. As the dilution rate is decreased, the oscillations disappear between a dilution rate of 0.11 and 0.12 hr$^{-1}$ and the culture remains in a steady state at the lower dilution rate of 0.1 hr$^{-1}$. The oscillations do not reappear as the dilution rate is changed back to its former value. ................................. 42
2.4 Time course of exhaust CO$_2$ signal for 3 different continuous cultures operated at the same dilution rate and medium feed composition.

3.1 Typical oscillation patterns associated with the 1:2 attractor (a) and 1:3 attractor (b). At $S_f = 30$ g/L and $D = 0.15$ hr$^{-1}$ a periodic solution that belongs to the 1:2 attractor is obtained. Oscillations are observed in all system parameters such as cell number concentration (a-1) and substrate concentration (a-2). The 1:2 attractor is characterized by one subpopulation of mothers (a-3) and two subpopulations of daughters (a-4). At $D = 0.095$ hr$^{-1}$ a periodic solution that belongs to the 1:3 attractor is obtained. Oscillations are also observed in all system parameters such as cell number concentration (b-1) and substrate concentration (b-2). The 1:3 attractor is characterized by one subpopulation of mothers (b-3) and three subpopulations of daughters (b-4).

3.2 Bifurcation diagram at $S_f = 30$ g/L, obtained by employing moderate intensity functions $\Gamma_{T1}$ and $\Gamma_{D1}$. Bifurcations are shown in terms of cell number concentration (a) and substrate concentration (b). The diagram shows maximum and minimum cell number and substrate concentration during the oscillations obtained from MATLAB (●), maximum and minimum cell number and substrate concentration during the oscillations obtained from the continuation code (- -) and the steady state profiles (—).  

3.3 The effect of the “sharpness” of intensity functions on the range of dilution rates supporting the 1:2 attractor. The curves show the maximum and minimum values of the substrate concentration during the oscillations under moderate intensity functions ($\Gamma_{T1}$ and $\Gamma_{D1}$) (—) and sharp intensity functions ($\Gamma_{T2}$ and $\Gamma_{D2}$) (—) parameter values.

3.4 Bifurcation diagram at $S_f = 30$ g/L, obtained by employing sharp intensity functions $\Gamma_{T1}$ and $\Gamma_{D1}$. The diagram represents the 1:1 and 1:2 attractors in terms of cell number concentration (a) and substrate concentration (b). The diagram shows maximum and minimum cell number and substrate concentration during the oscillations obtained from the continuation code(- -) and the steady state profiles (—).

3.5 The effect of feed substrate concentration, $S_f$, on the range of dilution rate supporting the 1:2 attractor. The curves show the maximum and minimum values of the substrate concentration during the oscillations at $S_f = 15$ g/L (—), 30 g/L (- -) and 60 g/L (• •).
3.6 Bifurcation dynamics of budding yeast investigated experimentally (a) indicate that an oscillatory state and a steady state can be achieved at the same operating conditions. Model simulations (b) predict the same bifurcation mechanisms. In the top figure, the actual response of CO$_2$ concentration in the exit gas stream (—) to set point changes in the dilution rate (- -) is shown. In the bottom figure, the corresponding simulated response of substrate concentration (—) to changes in dilutions rate (- -) is shown. ...............76

3.7 Results of model simulations performed at the operating conditions $S_f = 30$ g/L and $D = 0.14$ hr$^{-1}$ and starting with the corresponding steady state distribution as the initial condition. Model equations were integrated using MATLAB stiff ODE solver, ODE15s (a) and the continuation code ODE solver, ODESSA (b). The simulations show that the steady state can be observed for hundreds of virtual hours (note the range on the substrate concentration axis), however, simulations performed using ODE15s predict a stable steady state, while simulations performed using ODESSA predict an unstable steady state. ..........80

4.1 The Cell Cycle of $S$. cerevisiae ..........85

4.2 Flowchart illustration of the solution procedure for the hybrid mass-age distribution model ..................................................98

4.3 Comparison between the numerical evaluation of the analytical steady state distributions (●) obtained using a Mathcad program and the steady state distributions predicted by the hybrid mass-age distribution model simulation obtained using MATLAB code (—). The steady state is shown in terms of the cell mass distribution of unbudded cells (a) and the cell age distribution of budded cells (b). ....121

4.4 Comparison between the experimentally observed batch growth dynamics followed by a switch to the continuous operation at the stationary phase of the batch in terms of evolved CO$_2$ (a) and the model predictions of such dynamics in terms of the substrate concentration (b) and the cell number concentration (c) ........123

4.5 The bimodal cell size distribution of unbudded cells at the stationary phase of the batch growth observed experimentally (a) and predicted by the hybrid model (b) ..............124

ix

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
4.6 Typical oscillation patterns associated with the 1:5 attractor (a) and 1:3 attractor (b). At $S_f = 30$ g/L and $D = 0.065$ hr$^{-1}$ a periodic solution that belongs to the 1:5 attractor is obtained. Oscillations are observed in all system parameters such as substrate concentration (a-1) and cell number concentration (a-2). The 1:5 attractor is characterized by one subpopulation of budded cells (a-3) and five subpopulations of unbudded cells (a-4). At $D = 0.095$ hr$^{-1}$ a periodic solution that belongs to the 1:3 attractor is obtained. Oscillations are also observed in all system parameters such as substrate concentration (b-1) and cell number concentration (b-2). The 1:3 attractor is characterized by one subpopulation of budded cells (b-3) and three subpopulations of unbudded cells (b-4).

4.7 The existence of a stable oscillatory state and an extended unstable steady state at the same operating conditions. At $D = 0.095$ hr$^{-1}$, the model predicts autonomous sustained oscillations if the initial culture was a stationary phase batch culture (a), however, if the dilution rate was changed slowly from a value that does not support oscillations, at which point the culture is at steady state, to $D = 0.095$ hr$^{-1}$ an extended unstable steady state is predicted (b).
Abstract

The conditions that precede the onset of autonomous oscillations in continuous yeast cultures were studied in three different types of experiments. It was found that the final state of the culture depends on the protocol used to start-up the reactor. Reaching the desired operating point by slow dilution rate changes gave rise to different final states, two oscillatory states and one steady state, depending on the rate of change in dilution rate. The multiplicity of stable states at a single operating point is not explained by current distributed models and points towards a segregated mechanism of these oscillations.

The ability of an age population balance model to capture experimentally observed oscillatory dynamics of continuous cultures of budding yeast was investigated. Consistent with experimental evidence, numerical simulations of the model revealed the existence of several, stable periodic solutions. However, each occurred over a different range of dilution rates. Experiments have shown that the steady state in continuous yeast cultures appears to be stable, even under conditions that allow oscillatory dynamics. The stability of the steady state of the age population balance model under conditions that allow oscillatory dynamics was not resolved.

Another population balance model in terms of mass distribution of unbudded cells and age distribution of budded cells was also proposed. The model was based on a more detailed cell cycle than that used in the development of the age distribution
model. Therefore, the hybrid mass-age model was superior to the age model in its ability to simulate situations in which the yeast culture is starved. In agreement with experimental evidence, the model predicts the auto-synchronization of batch yeast cultures during their batch growth with a final bimodal cell mass distribution. Furthermore, the oscillations occur spontaneously as the simulated batch culture is switched to continuous operation. The model also predicts multiple oscillatory states at separate regions of the dilution rate and predicts the existence of an oscillatory state and an extended unstable steady state at the same operating conditions.
Chapter 1

Introduction

1.1 Literature Review

1.1.1 Autonomously Oscillating Yeast Cultures

The bakers' yeast *Saccharomyces cerevisiae* is an essential microorganism for many industries such as brewing, baking, and food manufacturing. Yeasts, in general, and *S. cerevisiae* in particular are also gaining increased importance in genetic engineering applications as well. Under specific operating conditions a continuous culture of *S. cerevisiae* experiences sustained autonomously occurring oscillations in the concentration of extracellular and intracellular parameters. Examples of these parameters are carbon dioxide evolution rate, oxygen uptake rate, glucose, ethanol and acetic acid concentrations, pH controller action, as well as biomass concentration, cell size distribution, protein content and storage carbohydrates [1, 4, 8, 9, 31, 34, 35, 36, 37, 38, 48, 49]. Three types of autonomous oscillations have been reported in literature [22, 23]: cell cycle dependent oscillations, glycolytic oscillations, and short-period-sustained oscillations. This work addresses only on the cell cycle dependent oscillations. A marked cell cycle synchronization of the yeast population is observed in association with these oscillations and strong interactions
between the synchronized cell cycle events and the oscillating metabolic signals are reported [31, 35, 36, 38, 48, 49]. Cell cycle dependent oscillations have been observed in glucose-limited environments and under aerobic growth conditions. These oscillations appear in intermediate dissolved oxygen levels [8, 31, 38] and for a range of dilution rates, typically 0.09-0.25 hr\(^{-1}\) [37] and they were hard to suppress in high performance chemostats at dilution rates between 0.13 hr\(^{-1}\) and 0.2 hr\(^{-1}\) [48]. The periods of oscillation varies from 2 to 45 hr depending on the particular strain and culture conditions [8]. This period is related but not equal, to the mass doubling time of the culture [38, 52]. Figure 1.1 shows a typical oscillation pattern of a continuous culture of *S. cerevisiae*. The oscillations are shown in terms of the variation in glucose and ethanol concentrations, exhaust carbon dioxide, and percentage of dissolved oxygen signals during two consecutive cycles.

Many biochemical and physiological studies were carried out to explain the cause of the autonomous oscillations and the stabilizing mechanisms for these process dynamics [1, 6, 8, 9, 21, 23, 31, 34, 35, 36, 48, 49, 52]. The mechanism that originates the oscillatory behavior is controversial and none of the models known to us satisfactorily addresses all aspects of the phenomenon [6, 36, 38, 48, 50]. An ideal model of a bioreactor microbial culture should account for the important metabolic pathways employed by the culture which dictate substrates consumption and products and biomass generation, the behavioral differences between the individual cells due to their varying physiological states, as well as the dynamic interaction between these
Figure 1.1: A typical oscillation pattern of synchronous culture of *S. cerevisiae*. in terms of glucose concentration, exhaust CO$_2$%, ethanol concentration and percentage of dissolved oxygen (saturation at 100%) during two consecutive cycles. The operating conditions are: dilution rate, $D = 0.15$ hr$^{-1}$, glucose feed concentration, $S_f = 30$ g/L, and pH = 5.0.

segregated cells. Fredrickson introduced the term "segregated", to indicate explicit accounting for the presence of heterogeneous individuals in a population of cells that may have very different chemical structure or state, and the term "structured", to designate a formulation in which cell material is composed of multiple chemical components [2, 26]. Segregated models are required to accurately describe situations in which chemical composition varies significantly between cells [45]. Nevertheless, segregated and structured mathematical models of microbial cultures are rarely en-
countered in literature probably due to the complexity of the associated parameter estimation problems as well as the inherent difficulty in solving the resulting equations. The less cumbersome unsegregated and/or unstructured models are usually proposed as descriptions of microbial cultures. Unsegregated models are based on the assumption that the biophase is well mixed and they represent the case where all the individual cells have the same properties and chemical composition [2]. Unstructured models, on the other hand, are based on the assumption that cell states can be represented by a single component, such as cell mass or cell age.

Studying the various factors affecting the cell cycle events and their duration as well as the major metabolic pathways available for \textit{S. cerevisiae} was at the heart of model development of oscillating yeast cultures. The following two sections summarize some of the important features of the budding yeast cell cycle and its metabolic behavior.

1.1.2 Cell Cycle of \textit{Saccharomyces cerevisiae}

The cell cycle of \textit{S. cerevisiae} has been traditionally studied as an example of eukaryotic cell cycle and asymmetric division. Even though, there is no such a thing as a typical eukaryote, studying the \textit{S. cerevisiae} cell cycle may reveal some information about eukaryotic systems [3]. It is generally believed that the molecular events which take place in \textit{S. cerevisiae} are likely to be relevant to the events occurring in eukaryotic cells of higher organisms [42]. The concept of the eukaryotic cell cycle was first introduced by Howard and Pelc nearly four decades ago [46]. Cells with
only one full genome are said to be in the presynthetic phase, G₁ [41]. During the DNA synthesis process and before the attainment of two full genomes the cells are said to be in the synthesis phase, S. After completing DNA doubling, cells with the double amount of nuclear DNA enter the postsynthetic, premitotic phase, G₂, and remain in this phase until the commencement of division in the mitosis phase, M [35]. A schematic diagram of the S. cerevisiae cell cycle is shown in figure 1.2. The cell cycle is characterized by a critical cell mass, $m_s$. Cells with mass greater than $m_s$ are called mothers, while cells with less mass are called daughters. The point of the cell cycle when a cell reaches $m_s$ is called the “Start” [15, 29, 48]. Cells at the “Start” grow for a period of time, $U_p$, before the emergence of a small bud. The cell mass at this point is referred to as the transition mass, $m_t$. The budded cell continues growing with most of the increase in mass taking place in the bud until the division point, $m_d$, is reached. This period is the budded phase period; B. At division the bud separates, forming a new daughter cell and the original mother cell is back to the “Start” point as a new mother. The mother cell generation time, $P_m$, is the time required for a mother cell at the “Start” to reach the division point. The division in S. cerevisiae is asymmetric and daughters are produced at smaller mass, $m_0$, than mothers are. Therefore, daughters have longer generation time, $P_d$, than that of the mothers. Asymmetric division approaches symmetric division only at maximum growth rates [15, 17, 18, 29]. Current evidence indicates that the S-phase starts approximately the same time as bud emergence as shown in figure 1.2 [17].
Figure 1.2: Schematic diagram of *Saccharomyces cerevisiae* cell cycle.

The cell cycle of exponentially growing cultures, the unequal division and the relation between the cell cycle parameters and the population growth rate were all extensively studied. The studies revealed that only a little increase in the budded interval, $B$, could be observed as the population doubling time increases. The unbudded interval for mother cells, $P_m$, increases moderately while the unbudded interval for daughter cells, $P_d$, increases considerably at slower growth rates. Since most of the growth at the budded interval is associated with the bud and also since the budded interval does not vary much with growth rate, poor nutritional conditions lead to formation of smaller daughters at cell division as compared to daughter cells formed in rich environment [15, 29]. The critical cell size for budding was not very sensitive to variation in the growth rate [15]. More recent studies produced correlations that reveal the yeast population mean parameters and the durations of the cell cycle phases under different growth rates [51, 29].
In a more recent work [1] it was reported that the yeast cell cycle is controlled by the attainment of two thresholds, namely, the critical cell mass required for budding $m_t$ and the critical cell mass required for division $m_d$. It was also observed that both $m_t$ and $m_d$ are affected by the nutritional conditions in the media and thus the growth rate. Poor nutritional conditions lowers both $m_t$ and $m_d$ with a higher impact on $m_d$. The ratio $h = \frac{m_d}{m_t}$ drops from around 1.6-1.7 at good nutritional conditions to 1.15-1.2 at very poor nutritional conditions [1, 31]. Münch (1992a), however, criticized this model and argued that the model is not true for all dilution rates and pH values. At a lower set of dilution rate and pH value than that reported in the previous studies [1, 31] daughter cells with heterogeneous cell size distribution separated from their mothers at the same instant. It was concluded that the bud size before separation was obviously not only dependent on the actual growth rate [6, 15], but could vary under constant growth conditions as well. It was found that the cell size development depends strongly on the growth environment: the kind and concentration of the substrate as well as physical/chemical conditions (pH for example) influence the quality and the dynamics of growth of a single cell. It was recommended that these observations should be taken into account whenever the cell size is used as a criterion in dynamic growth characterization.

As a result of the extensive study of S. cerevisiae cell cycle, there is currently a number of equations to calculate the duration of cell cycle events [15], volumes of cells during the cycle [51] and genealogical age distribution [29] from mean popu-
lation data. Hjortsø and Bailey (1982) have formulated and solved a population-balance mathematical model for the growth of *S. cerevisiae* cultures in well-mixed continuous-flow reactors, chemostats, utilizing a cell cycle model that incorporates asymmetric division. They have also illustrated a general approach for determining frequency functions for observable quantities by combining age-distribution results with single-cell kinetic models [17]. In addition, the asymmetric division cell cycle has been used in the formulation of a population-balance model of the transient dynamics of *S. cerevisiae* cultures. A stepwise solution of the system of equations has been illustrated elsewhere [18].

### 1.1.3 Metabolic Pathways in *Saccharomyces cerevisiae*

The yeast *S. cerevisiae* is fermentative yeast. It is able to utilize glucose under anaerobic and aerobic conditions which means in the absence and presence of oxygen, respectively. In the former case it converts glucose to ethanol and CO$_2$. During aerobic growth both fermentation and respiration may contribute to the conversion of glucose [42]. After a short lag, yeast batch cultures start an exponential growth phase with oxidative and fermentative degradation and consumption of glucose. This is concluded from the increasing demand for oxygen and the production of ethanol at the same time. Then, the so-called diauxic lag phase prevails which is characterized by a stagnation of biomass increase [34]. During this phase, the organism switch-over in its enzymatic make up, synthesizing new enzymes which results in an adaptation of the cells to consume the previously excreted ethanol purely
oxidatively. A considerable increase in the demand for oxygen is observed at this point. Ethanol concentration reaches its maximum level, as glucose depletes [35]. Figure 1.3 shows the characteristic pattern of glucose consumption, ethanol excretion, dissolved oxygen and dry weight variations in a batch culture. The diauxic lag phase cannot be seen from figure 1.3 since the lag phase period is of comparable length of the sampling time interval. In continuous cultures, the metabolic pathway for glucose is highly dependent on the specific growth rate, which equals the dilution rate at steady state. Under low dilution rates, the consumption of glucose is predominantly oxidative as evidenced by a respiratory quotient, RQ, of 1.0 [52, 21]. The respiratory quotient, RQ is defined as the ratio of carbon dioxide production rate to oxygen uptake rate. With increasing dilution rates a fermentative type metabolism arises as evidenced by an RQ > 1.0. [52, 35]. Kinetic studies on S. cerevisiae show that the maximum growth rate available from glucose fermentation is about 0.45 hr$^{-1}$, with a low cell mass yield of 0.15 g per g glucose consumed. In contrast, the maximum growth rate available from ethanol oxidation is about 0.20 hr$^{-1}$, with a high cell mass yield of 0.65 g per g ethanol consumed [21]. The growth rate available from ethanol oxidation is much slower than that available from glucose fermentation, however, the cell mass yield is much higher. During fermentation, much of the available glucose mass was excreted as ethanol, reducing the cell mass yield on this pathway, while all of the carbon consumed via ethanol oxidation pathway is used for the production of cell mass.
Figure 1.3: Glucose consumption, ethanol production and consumption, percentage of dissolved oxygen (saturation at 100%) and the dry weight concentration during a batch culture of *S. cerevisiae*.

The oxidative metabolic pathway of glucose consumption has been discovered in continuous culture experiments operated at low dilution rates where the residual glucose concentration in the chemostat is below about 50 mg/L. This metabolic pathway is repressed or inhibited at high glucose concentration (a phenomenon known as the Crabtree effect). Therefore, it is not commonly observed in batch cultures nor in chemostats operated at high dilution rates. Consequently, it has not been possible to accurately measure the maximum specific growth rate for the glucose oxidation pathway in either continuous or batch cultures. However, the cell
mass yield for this oxidative pathway, which is predominant at low dilution rates has been estimated at about 0.50 g per g glucose consumed [21].

### 1.1.4 Conceptual and Mathematical Models

Despite the uncertainty regarding the mechanism which causes the oscillatory behavior, many conceptual and mathematical models were proposed as explanations of the autonomous oscillations based on the available biochemical and physiological studies [8, 9, 21, 23, 31, 34, 35, 36, 43]. The resulting models can be conceptually classified into two categories, distributed, metabolic models and segregated models. Distributed models use variations and modifications of Monod’s classical model [33] for microbial growth in order to predict the oscillations. In these models the biomass phase is considered a well-mixed phase and the oscillations are not cell cycle dependent, instead, the oscillations occur due to bifurcations in the metabolic kinetics [21]. Distributed models cannot explain the observed cell cycle synchrony which is basically a segregated phenomenon. It is assumed, however, that the cell cycle synchrony is induced by the metabolic oscillations in a mechanism similar to induction synchrony. This explanation is, nonetheless, questionable because it is based on the unexplained coincidences that the period of the oscillations match the characteristic time of the cell cycle and that the metabolic oscillations have magnitudes that will result in induction synchrony. In segregated models, however, the biophase is not considered well mixed but segregated into individual cells that may have very different chemical composition or state. In segregated models, a partially
synchronized culture induces periodic changes in the medium composition and these changes in turn stabilize the cell cycle synchrony. The models explain why the oscillations are stable but do not explain what causes the initial synchrony that initiates the oscillations. In the following discussion, a cybernetic model is first reviewed as an example of distributed models. Then the most significant cell cycle models are visited and reviewed in as much chronicled order as possible.

Jones, K. D. and Kompala, D. S. (1999) have proposed a cybernetic model to describe the growth dynamics of *S. cerevisiae* in both batch and continuous cultures. They have proposed a structured unsegregated model, which included three competing metabolic pathways. These pathways are fermentative consumption of glucose, oxidative consumption of glucose and oxidative consumption of ethanol. All the metabolic pathways have been assumed available for the cell mass growth; however, the metabolic pathway with the highest growth rate dominates the growth. The advantage of using cybernetic modeling framework is that it replaces the detailed modeling of regulatory processes with the so called cybernetic variables which represent the optimal strategies for enzyme synthesis and activity [21]. Upon the incorporation of these variables in the biomass, substrates, storage carbohydrates and enzymes balances, they dictate the relative importance of each metabolic pathway and the production rates of the different key enzymes. The model predicts that, in batch cultures, the initially high glucose concentration favors the fermentative metabolic pathway and at the same time suppresses the oxidative pathway of glu-
cose consumption. As a result ethanol is produced, oxygen accumulates and glucose depletes. These new conditions lead to the induction of oxidative enzymes synthesis. Then, after a lag phase, a shift towards the oxidative pathways takes place, during which both ethanol and oxygen are depleted. These predictions are consistent with the experimentally observed sequential uptake of glucose then ethanol separated by the diauxic lag period. Model simulations of continuous cultures, at high dissolved oxygen levels, predict steady state behavior for a wide range of dilution rates starting at about 0.1 hr\(^{-1}\) up to the wash out dilution rate. In addition, the model predicts a critical dilution rate of 0.23 hr\(^{-1}\) around which a metabolic shift occurs. At dilution rate values lower than the critical point glucose consumption was predominantly oxidative in nature as evidenced by the chemostat lack of ethanol and the low exhaust CO\(_2\) evolution rate, beyond the critical point glucose consumption became fermentative and high values of steady state ethanol concentration and CO\(_2\) evolution rate were observed. This phenomenon is consistent with experimental observations and is attributed to the Crabtree effect.

Oscillations were experimentally observed in continuous cultures at intermediate dissolved oxygen levels [37]. In the development of the cybernetic model a set of four parameters was not amenable to experimental determination. Bifurcation algorithms were used to determine the ranges of these four parameters which would yield oscillations at low to intermediate dissolved oxygen concentration. A wide range of parameter values was found to produce oscillations. A specific set of these
parameters that presumably predict reasonable periods of oscillations was reported and used to illustrate the oscillatory dynamics. The metabolic feed-back loop for the cybernetic model was explained based on model simulation results [21]. Model simulations showed that the oscillatory dynamics prevail for a range of dilution rates and dissolved oxygen levels. The oscillations appear and disappear very quickly among the entrance to and the departure from the permissible range of operating conditions, respectively. This behavior is consistent with the experimental observations of Parulekar et al. (1986).

The cybernetic model suffers from a number of major drawbacks. First, since the model does not account for the culture segregation into individual and potentially different cells, it does not predict or even account for the observed autonomous cell cycle synchronization of the culture [6]. The authors' notion that cell cycle synchrony might be induced by the metabolic oscillations is questionable because it relies on the unjustified assumption that the period and magnitude of the metabolic oscillations is appropriate for induction synchrony. Second, the quick elimination and regeneration of metabolic oscillations in response to the changes in operating conditions was considered a strong evidence that the dynamic competition between the three metabolic pathways is indeed the causative factor for the oscillations. This is not necessarily true, it was observed that the metabolic oscillations predicted by the model not only appear and disappear quickly but also that the transients associated with these oscillations die out very quickly as they assume their final period and
amplitude. However, Fast Fourier Transform (FFT) analysis of experimental data from our lab indicate that the actual system has slow dynamics and the transients associated with the oscillations may take up to two days to vanish [5]. Furthermore, in all the experiments presented for comparison, the changes in the operating conditions were performed as step shifts in operating conditions. More recent data from our lab indicate that the final dynamic state attained by the culture depends not only on the initial and final operating conditions but also on the path pursued to move from one set of operating conditions to another. In agreement with previous investigations [38], our data indicate the existence of multiple oscillatory states and an extended non-oscillatory state at the same operating conditions, which is in direct contradiction with the cybernetic model predictions. Finally, the period and amplitude of the predicted oscillations changed considerably over a relatively small range of dilution rates which is inconsistent with experimental observations.

The work of von Meyenburg (1973) is among the first cell cycle dependent explanations of the oscillatory behavior of *S. cerevisiae*. In this original work it was suggested that the oscillations were caused by partial division synchrony, and that the sequence of storage and break down of reserve carbohydrates enables the cells to complete the important budding phase in a rather constant time independent of the extracellular conditions. It was also speculated that the pH control using NaOH could play an important role in maintaining synchrony by affecting Na$^+$ concentration in the culture. It is very unlikely, however, that the small changes in ion
concentrations inflected by the pH controller could have a sufficiently strong effect on the population to maintain synchrony [48]. The oscillation has also been observed in systems without pH control. In these systems there was a periodic change in the medium pH value [8, 9] which is in direct contradiction with von Meyenburg's suggestion. Porro et al. (1988) indicated that the oscillations exist only in a well defined range of dilution rates and dissolved oxygen values. They also observed that at high dilution rates two oscillatory regimens with different periods occur in different ranges of dissolved oxygen. Their analysis of budding index showed a marked degree of synchronization of the oscillatory cultures. Based on their observations and experiments they proposed the following hypothesis for synchronous growth. In a particular part of the cell cycle, just before budding, there is an increasing demand for metabolic energy. Under limited oxygen availability, a fully respiratory metabolism of glucose is not possible and therefore, the yeast produces ethanol. The produced ethanol is secreted and its presence modifies the environment of the cell population. In the presence of the poor nutrient ethanol, the setting of $m_c$ and $m_d$ is lowered. So, more cells are induced to bud; this creates a positive feedback loop that synchronizes a group of cells. Since this effect occurs at each cell cycle, a limit cycle is created and sustained oscillations appear [38]. In a more recent work of the same group [31], they reported that the production of ethanol is strictly coupled with the mobilization of internal carbohydrate reserves. They also modified their model for synchronous growth. In this work, it was proposed that after the excretion
of ethanol the yeast cells utilize both glucose and ethanol as a mixed substrate. The mixed substrate will lead to a higher growth rate under which both $m_t$ and $m_d$ increase. When ethanol is exhausted, a nutritional shift down occurs, with nutritional imbalance between the higher biomass concentration and the limited glucose availability. This has two consequences: First, the growth rate decreases and second, the ratio $h$ drops to its original value before the mixed substrate growth. These two effects cause a strong decrease of the budding index, because the decrease of $h$ forces a fraction of the budded cells to undergo cell division. This in turn will establish a positive feedback loop and maintain the oscillations.

An important structured and segregated mathematical model of S. cerevisiae continuous cultures was proposed by Strässle et al. (1988, 1989). In this model the population was discretized by cell mass into a number of classes. Mother and daughter cells were identified by comparing cell mass to the cell mass required to form a bud. The model was based on a number of assumptions and experimental observations such as the respiratory bottleneck hypothesis [48], accumulation of internal storage carbohydrates during the single cell phase and mobilization of these reserves during the budding phase, constant duration of the budding phase and asymmetric division. At one point in the cell cycle, mother cells start to mobilize internal storage carbohydrates. This additional internal substrate flux leads to a temporary overflow of the respiratory capacity of the budding cells and results in excretion of ethanol. Non-budding cells are able to consume this excreted ethanol and increase
their growth rate temporarily. An amplifying effect on random fluctuations in the age distribution of the culture might therefore take place and an acceleration of single cells ready for the next cell cycle step would result. A continuous polarization of subpopulations would result and end up in spontaneous synchronization.

Cazzador et al. (1990) proposed another structured and segregated model. In this model, the population was also discretized in terms of cell mass into several mass compartments and the cells were segregated into mothers and daughters. The model was based on the observation that cells at the beginning of the DNA synthesis phase utilize storage carbohydrates resulting in excretion of ethanol. The model was also based on the physiological studies conducted by Porro et al. (1988) and Martegani et al. (1990). In this model, it was proposed that the yield coefficient for daughter cells is different from that associated with mother cells.

Although both are structured and segregated models, it should be noted that the models proposed by Sträßle et al. and Cazzador et al. are inherently different. For example, the former model assumes that the budding interval is constant, while the latter assumes that only the critical mass for budding is constant while the critical mass for division is a function of substrate concentration. Furthermore, the model proposed by Sträßle et al. suggests different metabolic behavior of the budded and unbudded cells while the model proposed by Cazzador et al. assumes that the growth rate for all the cells is the same and is governed by the Monod form, with the unverified assumption that the metabolisms of daughter and mother cells
represented by the yield coefficients are different. Computer codes were used to solve the discretized forms of both models. Both models could predict some characteristics of the oscillatory dynamics of the system, however, several discrepancies between model predictions and experimental observations were reported [6, 49]. A major disadvantage of the model introduced by Cazzador et al. lies in the utilization of the unjustified assumption that daughter cells differ from mother cells in their yield coefficients. On the other hand, a major disadvantage in Strässle et al.'s model lies in the use of the bottleneck assumption which has been contradicted by many experimental studies [21]. The solutions of the models were laborious due to the requirement of large number of discrete classes to obtain solutions in good agreement with the experimental results. Model parameters which were not possible to determine experimentally were estimated by fitting experimental data. However, the validity of these parameters, is questionable, which is an inherent problem in all structured models.

Münch et al. (1992) presented a quantitative overview of the cell proliferation processes in different transient experiments [35]. They have criticized previous models which explain the spontaneous synchronization phenomena on the basis of slow continuous polarization process of subpopulations [48, 49] or passing over a sharp threshold value [6, 31]. They found out that spontaneous oscillations appear very quickly after entering into the permissive conditions for synchrony. They also observed that cells at a large time window of the cell cycle are potentially ready to start
a new proliferation step after establishing permissive conditions. Also the spontaneous synchrony relaxes immediately by moving out of the permissive region, with no memory effects exist to sustain synchrony. They also argued that even structured segregated models are incapable of explaining transient behaviors because they do not consider cell cycle dependent, physiological properties of different cells as model variables [35].

Hjortsø and Nielsen (1994, 1995) proposed a conceptual segregated unstructured model for the oscillating yeast cultures. A feedback loop for the maintenance of cell synchrony was proposed as follows. A partially synchronized culture will produce periodic changes in the medium as they pass through their cell cycles. This, in turn, results in a periodic forcing of the population balance equation that may result in a stabilization of the cell synchrony [19]. The cell cycle in this model contains two relevant control points: the transition age, $a_t$ and the division age, $a_d$. At age $a_t$, daughter cells attain the same state as that of the newborn mothers. At this point they become mothers and combine with the newborn mothers and their ages are reset to zero. The mother cells continue growing until they reach the age $a_d$ at which they divide (see figure 1.2). Both these ages are functions of the medium composition and other environmental parameters [20]. It must be emphasized here that in contrast to the discrete segregated models [6, 48, 49] the age distribution of cells in this model is a continuous function through the whole cell cycle. The segregation of cells into mothers and daughters is just a matter of mathematical
convenience and does not imply discretization of the model. A qualitative diagram
of the time-age trajectories is shown in figure 1.4. This diagram shows a special case
in which there is one subpopulation of mothers and one subpopulation of daughters
that are in perfect synchrony. In this case, both $a_t$ and $a_d$ are equal to the period
of oscillation, $P$.

Figure 1.4: Trajectories in the time-age plane of perfectly synchronized subpopulations of mothers and daughters.

To model the cell culture shown in figure 1.4 a population balance for each
subpopulation over the period of oscillation, $P$, is needed. These population balances
are algebraic equations relating the number of cells in each subpopulation to the
factor $\exp(-DP)$ which represents the fraction of cells not washed out. The model
also includes a differential equation for the substrate balance and some functions relating the ages at division and transition to the substrate concentration with some time delay. The model equations are subject to periodicity of period $P$ in the substrate concentration and cell number balances as boundary conditions over division and transition points. Furthermore, in order for the solution to be valid it must satisfy the following conditions: substrate and cell number concentrations must be positive and the division and transition ages must be decreasing functions of time during divisions and transitions [19, 20].

It should be noted here that the model predicts the existence of an infinity of periodic attractors with different subpopulation structures. The exact form of cell balances is determined solely by the structure of the attractor. In the terminology of Hjortso and Nielsen (1994, 1995), a structure that consists of $N$ mothers and $M$ daughters subpopulations is referred to as an $N:M$ attractor. The form of these balances is not, however, in any way dependent on the values of the model parameters. The same can, therefore, be said about the solution to these balances: The ratio of the cell numbers in two synchronized subpopulations is constant. This ratio would always involve the factor $\exp(-DP)$, which in turn will be constant. This leads to a very important result, for every attractor, the period of oscillation is inversely proportional to the dilution rate and it is dependent only on the structure of the attractor but not on the system parameters and therefore not on the single cell metabolism. Different attractors yield different oscillatory behaviors, it has been
pointed out that the simple attractors, such as 1:1, 1:2, and 2:1 attractors, are more likely to represent the experimentally observed oscillations [19, 20]. A solution based on a 2:1 attractor could fit experimental data obtained from literature [38], which implies that the model, though simple, has good potential [20]. Table 1.1 shows the model equations for the special case shown in figure 1.4 of the 1:1 attractor. Hjortsø and Nielsen (1994,1995) presented the analytical solution for this case and illustrated the general solution procedure.

Table 1.1: Model equations for the special case shown in figure 1.4

<table>
<thead>
<tr>
<th>Population balances for mothers and daughters:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W_m(a, t) = N_m\delta(t - a)e^{-Dt}$</td>
</tr>
<tr>
<td>$W_d(a, t) = N_d\delta(t - a)e^{-Dt}$</td>
</tr>
<tr>
<td>0 ≤ t ≤ P</td>
</tr>
</tbody>
</table>

Substrate balance:

$\frac{dS}{dt} = D(S_f - S) - \int_0^t \kappa W_d(a, t) - \int_0^t \kappa W_m(a, t)$

Age of devision and age of transition:

$\alpha_d = \pi_0 + \pi_1 \frac{\tau}{\tau - \tau}$

$\alpha_t = \pi_2 \frac{\tau}{\tau - \tau}$

Where $W(a, t)$ is the cell age distribution for cells of age $a$ at time $t$, $N$ is the cell number concentration, $S$ and $S_f$ are the substrate and feed substrate concentrations, $\kappa$ is the specific rate of substrate consumption divided by the yield and is constant, $\tau$ is the delay constant and $\pi_0$, $\pi_1$ and $\pi_2$ are constant model parameters. Subscripts $m$ and $d$ refer to mothers and daughters, respectively.

The final state of the system depends on the initial distribution of states since the culture should be at least partially synchronized to enter the feedback loop. The solution also depends on the effect of the environmental variables on both the transition age and the division age. The model parameters have not been fit to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
experimental data since the purpose of that work was to introduce the concept of the model and to illustrate the solution procedure for a simple example. The model shows that stable oscillations are not specific to a given compound or to a specific kinetic expression but can appear as a result of a feedback loop involving any compound that satisfies some very general kinetic requirements. The concentration of the other compounds in the system which are not included in the model are still most likely to be driven to oscillate due to the oscillatory behavior of the culture; thus, the oscillation in the concentration of certain compounds does not necessarily imply a causative link in the loop [20]. As a last remark, the model in hand is a very general model and many of the models present in literature can be obtained as special cases of this model by discretizing the distribution of states and/or by identifying which compound make up the feedback loop [20].

1.2 Statement of the Problem

Segregated and distributed modeling approaches rely on fundamentally different reasoning of the causative link and the stabilizing mechanism of the autonomous oscillations in yeast. The age distribution model suggested by Hjortsø and Nielsen (1994,1995) strongly suggests that a partial cell cycle synchrony is needed for the oscillations to commence. It is assumed that a cohort of synchronous cells would introduce periodic changes in the medium composition as they pass in union through the different events of their cell cycle. These periodic changes in the environment would then enforce the cell cycle synchrony and further stabilize the autonomous
oscillations. The models explain why the oscillations are stable but do not elucidate the cause of the initial partial synchrony that is required for the oscillations to begin. However, the cell cycle of *S. cerevisiae* is controlled in such a way that cells arrest in the G1-phase as nutrients are depleted, providing a possible explanation of why oscillations may start when a late batch culture is switched to continuous operation. Furthermore, any continuous culture that is not in a steady state is partially synchronized and even a slight deviation from a steady state distribution of states may be sufficient for oscillations to start.

Distributed, metabolic models, on the other hand assume that the oscillations occur due to the competition between the different metabolic pathways which result in a bifurcation of the model equations from a steady state to an oscillatory state. These metabolic oscillations are then responsible for inducing the observed cell cycle synchrony; however, this synchrony is not necessary for the initiation of the oscillations. This understanding is based on an unexplained coincidence that the period of the metabolic oscillations is of the same magnitude as the characteristic time of the cell cycle, and that the period and the amplitude of the metabolic oscillations are sufficient to produce the observed synchrony.

In this study, one of the objectives was to establish the necessity of the initial synchrony of the culture on the appearance of the oscillations in continuous flow reactors. Towards this endeavor, a number of experiments were conducted. In these experiments, the operating conditions of a chemostat were brought to a point that
is known to support oscillatory dynamics starting with different initial conditions in terms of the cell number distribution. Batch cultures of *S. cerevisiae* progressively synchronize into a bimodal cell size distribution during the course of their batch growth. In a set of experiments, the onsets of the continuous operation were chosen to be different phases of the batch growth. In another set of experiments, an initially synchronized culture was perturbed by mixing it with an externally grown randomized cell inoculum (RCI) before the start of the continuous operations. It was concluded that a disturbance of the initial partial synchrony of the culture may lead to the attainment of non-oscillatory dynamics at the same operating conditions that support oscillatory dynamics indicating that the initial cell culture distribution indeed affects the final dynamic state of the continuous culture.

A large number of the proposed mathematical models predict oscillatory dynamics of adequate periodicity and amplitudes. Nevertheless, these models differ significantly in their basic assumptions and the mechanism originating the oscillations. The recent advances in the methods and concepts of nonlinear mathematics makes these model more amenable to bifurcation analysis. In this study, it is aimed to develop a systematic tool for model discrimination by means of comparing the experimentally observed bifurcation phenomena to the bifurcation dynamics predicted by the mathematical model. Towards accomplishing this task a number of experiments were conducted to investigate the actual bifurcation dynamics of the system using the dilution rate as the bifurcation parameter. Our experiments confirm the
existence of multiple oscillatory states and an extended non-oscillatory state at the same operating conditions. A large number of distributed models that bifurcate through Hopf bifurcation do so in a such a way that as the bifurcation parameter crosses the bifurcation point the steady state becomes unstable and the oscillations spontaneously occur, independent of the path pursued or the rate at which the bifurcation parameter was changed. It was found experimentally, though, that the rate at which the dilution rate is changed from a point that does not support oscillatory dynamics to another that does, directly affects the dynamics at the final operating conditions. This observation suggests rejection of distributed models that do not predict this behavior such as the model proposed by Jones and Kompala (1999).

Chapter 2 features a detailed discussion of the experimental investigations carried out in this study and highlights the most significant results and conclusions.

In this work, an age distribution population balance model was developed and analyzed. The model was based on the original age distribution model suggested by Hjortso and Nielsen (1994, 1995). The new model, however, relaxes the simplifying assumption that cell transitions and divisions occur at discrete ages known as control points. Instead, cell transitions and divisions are governed by intensity functions, also known as breakage functions in other particulate systems applications [39]. These functions allow the transition and division events to take place over a range of ages which is biologically more reasonable. Previously, a hard time delay was used to account for the time lapsed between the changes in environmental con-
dition and cell response to these changes. The use of hard time delays is not suited for control purposes. Therefore, cells were forced to respond to a filtered substrate signal instead of a delayed signal through the use of the adaptivity concept. The model equations were solved using the method of orthogonal collocation on finite elements. Model simulations show the existence of multiple oscillatory solutions. However, these oscillations do not occur at the same operating condition, but at separate ranges of dilution rate. The model also predicts the existence of an oscillatory state and an extended steady state at the same operating conditions. There is conflicting evidence about the stability of this steady state, though. The bifurcation analysis strongly suggests that the oscillations emerge in this model due to a Hopf bifurcation and hence indicating the instability of the steady state at the operating conditions supporting the oscillations. Nevertheless, this model predicts very slow system dynamics, therefore, the steady state despite its instability would persist for considerably long durations and for all practical purposes it can not be distinguished from the experimentally observed extended non-oscillatory state. The model development, method of numerical solution, simulation results and bifurcation analysis are all illustrated in chapter 3.

The age distribution model was based on a simplified cell cycle with two relevant control points, the age of transition, $a_t$, at which daughter cells become mothers and their age is reset to zero, and the age of division, $a_d$, beyond which mother cells divide and give rise to a new mother cell and a new daughter cell both at age zero. In reality
the cell cycle is controlled by slightly different control points. The actual cell cycle is controlled by the attainment of the transition mass, $m_t$, beyond which budding can commence, and the attainment of the age of division or the fulfillment of the budded period, at which point cells start to divide. These two control points, the mass of transition, $m_t$, and the age of division, $a_d$, suggest that a population balance model that is based on the mass distribution of unbudded cells and the age distribution of budded cells, should be more biologically reasonable than is a model based purely on age or mass distributions of the whole population. As a matter of fact, the age distribution model is only valid as long as the simplified description of the cell cycle is valid. In the simplified cell cycle, $a_t$, corresponds to the mass at the start, $m_s$, while $a_d$ corresponds to the mass of division, $m_d$. However, there is no control point in terms of age that corresponds to the transition mass, $m_t$, as shown in figure 1.2. In other words, in the age distribution model there is no distinction between a budded and an unbudded mother cell. At very poor nutritional condition, this distinction becomes vital. When the substrate concentration diminishes, for example at the stationary phase of a batch culture, unbudded mother cells stop growing and never start budding unless the prevailing conditions change. Budded cells, on the other hand, are committed to divide; therefore, after some duration of time, budded cells divide irrespective of the environmental conditions. This behavior cannot be captured by the age distribution model and, therefore, it is not suited for situations at which starvation may occur.
In chapter 4, a hybrid mass-age population balance model is introduced. This model is based on a more detailed cell cycle than the one used for the development of the age model. The model uses mass as the internal state for unbudded cells and age as the internal state of the budded cells. The mass of transition is assumed constant, while the age of division was distributed over an interval of cell ages and hence the mass at division was, in turn, distributed over an interval of cell masses. Cell division in this interval was governed by a division intensity function. The partitioning of newborn cells into daughters and mothers is achieved by the means of a birth probability function. The method of orthogonal collocation on finite elements is invoked to numerically solve this model. Model simulations predict the existence of multiple oscillatory solutions at separate ranges of dilution rate. The model also predicts the existence of both an oscillatory state and an extended unstable steady state at the same operating conditions. The model development and a detailed illustration of the method of numerical solution along with simulation results are all presented in chapter 4.

The last chapter of this dissertation summarizes the most vital experimental observations and the conclusions drawn from these investigations. Brief comments in regard to the proposed population balance models, their features and their most significant predictions are discussed. Finally, recommendations and suggestions for future work are highlighted.
Chapter 2

Multiple Stable States and Hysteresis in Continuous, Oscillating Cultures of Budding Yeast

2.1 Introduction

Continuous cultures of bakers' yeast *Saccharomyces cerevisiae* can exhibit stable oscillatory dynamics in association with some degree of cell cycle synchrony under aerobic glucose-limited conditions [1, 9, 8, 31, 34, 35, 36, 37, 38, 48, 49, 4]. Extracellular and intracellular parameters, such as the concentrations of evolved carbon dioxide, dissolved oxygen, glucose, ethanol, pH controlling agent, storage carbohydrates, protein content, and cell mass as well as the budding index, exhibit oscillatory behavior. These oscillations are referred to as cell-cycle dependent [22] as opposed to other types of observed oscillations that do not result in cell cycle synchrony [22, 23, 14, 43]. The complex dynamics of the sustained oscillatory state may impose severe problems in process control [31, 38, 53].

Many experimental studies have been carried out to investigate the cause of the autonomous oscillations and the stabilizing mechanisms for these process dynamics [1, 9, 8, 31, 34, 35, 36, 48, 49, 22, 6]. Both conceptual and mathematical models were proposed based on these observations [31, 38, 48, 49, 6, 19, 20, 21, 25]. The proposed
mechanisms fall into two general classes, segregated models in which interactions between the cell cycle dynamics and the medium components cause cell cycle synchrony, and distributed, metabolic models in which the metabolic kinetics bifurcate to cause oscillations. Distributed models cannot explain the observed cell cycle synchrony which is an inherently segregated phenomenon. However, assuming that the period of the oscillations are of the same magnitude as the characteristic time of the cell cycle, the cell cycle synchrony can be explained as a result of induction caused by the metabolic oscillations [16]. This explanation does rely on an unexplained coincidence, that the metabolic oscillations have periods and magnitudes that will result in induction synchrony. Segregated mechanism of oscillations have been proposed using various models of single cell kinetics but no special assumptions are in fact needed since oscillations can occur even in an age distribution model [19, 20].

In segregated models, a partially synchronized culture induces periodic changes in medium composition and these changes in turn stabilize the cell cycle synchrony. The models explain why the oscillations are stable but do not explain what causes the initial partial synchrony that is required for the oscillations to begin. However, the cell cycle of *S. cerevisiae* is controlled in such a way that cells arrest in the G1-phase as nutrients are depleted, providing a possible explanation of why oscillations may start when a late batch culture is switched to continuous operation. Furthermore, any continuous culture that is not in a steady state is partially synchronized...
and even a slight deviation from the steady state distribution may be sufficient for oscillations to start.

Autonomous oscillations in budding yeast were first discovered at a time when the methods and concepts of nonlinear mathematics were less known in the scientific community than they are today [12]. In earlier work, model oscillations were therefore considered adequate for verification of the model. Only later were models proposed that described more detailed aspects of the oscillatory dynamics. However, the large number of proposed models, which in general do not agree about the mechanism, show that it is easy to find a model that exhibits oscillations and does a reasonable job of describing the changes in concentrations of various parameters during the oscillations. Little work has actually been done to try and systematically test these models with the aim of eliminating those mechanisms that cannot match experimental results. One way of doing such tests is to compare the experimentally observed bifurcation behavior with that predicted by the model. For instance, if model oscillations occur due to a Hopf bifurcation, then the steady state becomes unstable at the bifurcation point and a steady state should not be experimentally attainable under operating conditions that support oscillations. There have been reports of existence of a steady state and an oscillatory state at the same operating conditions [9], a phenomenon that cannot occur in a Hopf bifurcation. Confirmation of different experimentally obtainable states, be they steady states or oscillatory
states, is a powerful way of discriminating between classes of models since it is usually obvious whether or not a system oscillates.

In this work, we have investigated systematic methods for attaining a steady state under operating conditions that support stable oscillations. We found that a steady state is indeed possible, which indicates that mechanisms that rely on the spontaneous bifurcation of the steady state to attain oscillations cannot be correct, eliminating a substantial number of proposed mechanisms. We also found that at least three states, two oscillatory states and a steady state, are possible under identical operating conditions, further illustrating the complex bifurcation behavior of these cultures and limiting the number of possible mechanisms.

Three different strategies were tested for their ability to reach different states, 1) Addition to the fermenter of a randomized cell inoculum (RCI) isolated from shake flask cultures, 2) A switch from batch to continuous culture at different times of the batch growth curve, 3) Slow changes in dilution rate from one operating point to another.

2.2 Materials and Methods

The microorganism used in this study was *Saccharomyces cerevisiae*, H1022 (ATCC 32167). The strain was kept on agar slants at 4°C. Inoculum was prepared by transferring a single colony from the slant to a flask containing 30 ml YEP (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 0.1 ml PPG 2000). The inoculum
was grown in a shaker (Thermolyne) for 22 hours at 30°C and 180-rpm. The culture was inoculated on a 6% (v/v) basis.

Batch and continuous cultures were run in a Bioflo 3000 (New Brunswick) fermenter with a working volume of 1.0 L using medium D with increased concentrations of salts and vitamins [49]. It contained 30 g/L glucose, 9 g/L (NH₄)₂SO₄, 2.88 g/L (NH₄)₂HPO₄, 1.32 g/L KCl, 0.69 g/L MgSO₄·7H₂O, 0.42 g/L CaCl₂·2H₂O, 3.51 mg/L CuSO₄·5H₂O, 21.6 mg/L FeCl₃·6H₂O, 15.8 mg/L MnSO₄·H₂O, 13.5 mg/L ZnSO₄·7H₂O, 45 mg/L biotin, 90 mg/L m-inositol, 45 mg/L Ca-pantothenate, 9 mg/L thiamine, 2.25 mg/L pyridoxal-hydrochloride, 0.1 ml/L PPG 2000 and 0.7 ml/L sulfuric acid. Glucose, PPG and sulfuric acid with 80% water were autoclaved for 30 minutes at 121°C. The water used in the preparation of the glucose solution is pre-autoclaved for one hour before the addition of glucose, PPG and sulfuric acid. Vitamins, salts and the remaining water were filter sterilized using 0.22 μm Millipore filters. The water used in the preparation of the salt and vitamin solutions was autoclaved for 30 min and then cooled to room temperature prior to use. The temperature was maintained at 30°C and agitation rate was set to 800 rpm. The pH was kept at 5.0 using 4N NaOH. The culture medium was aerated with a flow of 1.5 SLPM. A DO probe (Ingold) and pH electrode (Ingold) measured dissolved oxygen and pH, respectively.

The signals from the Bioflo 3000 were collected through an A/D converter, monitored and recorded on a PC using Biocommand software (New Brunswick). The gas
analyzer for online measurements of exhaust oxygen, carbon dioxide and ethanol was Industrial Emission Monitor Type 1311 (Brüel & Kjær). The measurements were also collected through an A/D converter, and were monitored and recorded using the same software.

Two to three ml samples for the offline analysis were taken from the effluent of the fermenter and were filtered through 0.22 \( \mu \)m filters (Nalgene). They were then dried at 55°C until constant weights were achieved. Filtrate was kept at 4°C for further analysis. An HPLC (Shimadzu LC-600) equipped with Brownlee polypore H column operating at 75°C with a flow of 0.3 ml/min of 0.01N H\textsubscript{2}SO\textsubscript{4} as the mobile phase was used for ethanol, glucose and acetic acid measurements. A software package (Shimadzu EZChrom) was utilized for the data acquisition. Ethanol percentages in the exhaust stream were also calibrated with ethanol concentration in the liquid phase in g/L. This enabled us to correlate the online data obtained from the gas analyzer to the concentration of ethanol in the medium.

Cell number and cell size distributions were obtained using an Elzone Counter with a 48-\( \mu \)m orifice, calibrated with a mixture of 5, 9.9 and 20-\( \mu \)m latex beads. Samples of the culture were mildly sonicated and appropriately diluted with 3.1\% (w/v) NaCl solution prior to counting.

An external scale was fixed to the wall of the fermenter and calibrated to read the volume of the liquid phase. The mass of the feed vessel was measured and collected in a timely manner using an external balance. The feed mass flow rate was
determined as the rate of change of the feed vessel mass. The feed volumetric flow rate was directly calculated knowing the density of the feed media. The reported dilution rate was calculated as the ratio of the feed volumetric flow rate to the liquid phase volume in the fermenter. Due to the slight changes in the feed flow rate over extended periods of operation and the difficulty in controlling the level of the liquid phase, it was estimated that the absolute error in the reported dilution rates is in the order of ±0.005 hr⁻¹.

2.2.1 Preparation of Randomized-Cells Inoculum (RCI)

A colony from an agar slant was transferred into 20 ml YEP medium and grown at 30°C and 180 rpm in a Thermolyne bench top shaker for 22 hours. The resultant liquid inoculum was then used in the rest of this procedure to assure homogeneity. 50-ml centrifuge tubes were filled with 25 ml of fresh YEP medium and inoculated with the homogeneous liquid culture on a 2% (v/v) basis. These tubes were placed into the shaker at 30°C and 180-rpm. At different phases of batch growth, centrifuge tubes were collected and 0.1% (v/v) of 10% NaN₃ was added to stop their growth. They were then kept at 4°C. The cells were harvested by centrifuging the tubes at 6000 rpm and room temperature for 5 minutes. Cell pellets were re-suspended in 1 ml of YEP and combined together aseptically to form the corresponding RCI.
2.3 Results

2.3.1 Randomized Cell Inoculum

The objective of this study was to ascertain that a steady state is possible in a continuous culture under the same operating conditions where oscillations usually prevail. Externally grown cells were added to a culture at the time of switching from batch to continuous mode. The culture to which the externally grown cells were added was obtained either as an ordinary batch culture or as a continuous culture that had been temporarily switched to batch mode. The results for the latter are shown in figure 2.1. The initial continuous culture is clearly oscillating, as evidenced by the CO$_2$ signal. Between 8.5 and 26 hours, the culture is operated in batch mode after which a randomized cell inoculum is added and the system switched back to continuous mode. The RCI contained approximately $115 \times 10^8$ cells or about 5% of the total number of cells in the fermenter. Ethanol was spontaneously produced and its concentration reached a maximum value of 1.26 g/L. The production and subsequent depletion of ethanol took place within 4 hours. The culture appeared to start to oscillate but the oscillatory behavior disappeared after few irregular oscillations. Another ethanol production phase was observed after 12 hours, and the ethanol level reached a value as high as 2.8 g/L. There was no sign of any oscillatory behavior during the next 38 hours following the exhaustion of ethanol. Fast Fourier Analysis of the CO$_2$ data confirmed that the variations in this signal were not periodic but purely noise. Throughout the entire non-oscillatory state, the
level of dissolved oxygen was in the range of 55-65% of saturation. This, in fact, is the average level of dissolved oxygen observed in the oscillatory states. In order to eliminate the possibility that this behavior was due to accidental deficiencies in the feed, the culture was refreshed by increasing the feed flow rate to a high value for a short period of time (< 1.0 hour). The system was then switched to the batch mode for 18 hours, and then switched back to the continuous mode with the same feed and under the same operating conditions. A small amount of produced ethanol was depleted within 3 hours and sustained autonomous oscillations spontaneously appeared.

![Graph of exhaust CO2 and ethanol concentration](image)

**Figure 2.1:** Time course of exhaust CO2 and ethanol concentration with addition of externally grown cells. An initially oscillating culture is switched to batch at 8.5 hr and back to continuous mode at 26 hr. At this time, indicated by the vertical arrow, a randomized cell inoculum is added. After a few oscillations non-oscillatory behavior was obtained under the same operating conditions where oscillatory behavior was observed previously. The non-oscillatory behavior was not due to imperfections in the feed as evidenced by the oscillations obtained after switching the system to the batch (105 hr) and then to the continuous mode again (123 hr).
A similar, non-oscillatory state was obtained in the experiments where the externally grown cells were added to a late batch culture before switching this to continuous mode (data not shown).

The cell size distributions of the cultures in the fermenter and in the externally grown cells were also measured but the size distributions were in general similar and did not show special characteristics.

2.3.2 Switch Experiments

In these experiments, batch cultures were switched to continuous operation at different stages of their growth phases. Four specific growth phases were chosen for the start of the continuous culture operation, the early lag, exponential, diauxic lag and stationary phases or 2, 5.5, 7.5 and 22 hours after inoculation, respectively. The operating conditions of the batch (pH, temperature, aeration and agitation) were maintained after the switch to continuous culture (dilution rate of 0.15 hr\(^{-1}\) and glucose feed concentration of 30 g/L before sterilization). In all the experiments, a batch-like growth dynamics were observed. Results for the switch in early lag are shown in figure 2.2. Initially, glucose was consumed through the oxidation and fermentation pathways due to its high initial concentration. Ethanol was produced and accumulated to a maximum level in the range of 8.1-9.4 g/L during this phase. The relatively high concentration of ethanol suppressed any further production of ethanol. Glucose and ethanol were then consumed oxidatively which was inferred from a sharp decrease in the dissolved oxygen signal. The oscillations appeared
only upon depletion of ethanol regardless of the cell number concentration and dissolved oxygen levels. Cell number concentrations and dissolved oxygen levels were in the ranges of $(3.7 - 5.5) \times 10^{11}$ cell/L and 20-50%, respectively, just before the appearance of the first oscillation.

![Figure 2.2](image)

Figure 2.2: Progression of exhaust CO$_2$ and ethanol concentration for the experiments when the switch to the continuous operation took place at early lag. The vertical arrow indicates the time at which the culture was switched from batch to continuous mode.

2.3.3 Ramp Experiments

In these experiments, the dilution rate was changed slowly between two operating points. The CO$_2$ signal during a slow decrease in dilution rate followed by a slow increase is shown in figure 2.3. The culture is initially in a stable oscillatory mode at a dilution rate of 0.125 hr$^{-1}$. As the dilution rate is decreased, the oscillations disappear between a dilution rate of 0.11 and 0.12 hr$^{-1}$. The dilution rate is maintained at 0.10 hr$^{-1}$ for approximately 50 h during which time the culture reaches a
steady state. The dilution rate is then increased to 0.13 hr$^{-1}$ in a slow ramp, but the oscillations do not reappear. The reason that the final constant value of the dilution rate is not equal to the initial constant value is due to the difficulty in setting the dilution rate with high accuracy. The mechanical properties of the silicone tubing used for the feed stream changes with continued use and the working volume in the fermenter changes slightly with the state of the culture, addition of antifoam and pH controlling agent.

Figure 2.3: Time course of the exhaust CO$_2$ concentration during two slow ramp changes in dilution rate. The culture is initially in a stable oscillatory mode at a dilution rate of 0.125 hr$^{-1}$. As the dilution rate is decreased, the oscillations disappear between a dilution rate of 0.11 and 0.12 hr$^{-1}$ and the culture remains in a steady state at the lower dilution rate of 0.1 hr$^{-1}$. The oscillations do not reappear as the dilution rate is changed back to its former value.
The ramp experiments gave different final states, dependent on the rate at which the dilution rate was changed. A step change from a batch stationary phase culture to a dilution rate of 0.15 hr\(^{-1}\) gave the commonly observed oscillations, figure 2.4a. A 24 hr ramp from a steady state culture at a dilution rate of 0.10 hr\(^{-1}\) to a dilution rate of 0.16 hr\(^{-1}\) gave oscillations of a different period and with a qualitatively different shape, figure 2.4b, while a 48 h ramp from a steady state at a dilution rate of 0.10 hr\(^{-1}\) to a dilution rate of 0.15 hr\(^{-1}\) gave a steady state, figure 2.4c. Presumably, in the last experiment, the rate of change in dilution rate was small enough for the culture to remain in a quasi steady state.

Figure 2.4: Time course of exhaust CO\(_2\) signal for 3 different continuous cultures operated at the same dilution rate and medium feed composition.
2.4 Discussion

The existence of both a steady state and an oscillatory state at the same operating condition is documented by both the ramp experiments and the experiments in which RCIs were added to a fermenter. This result invalidates mechanisms which oscillate due to a supercritical Hopf bifurcation, the simplest and probably most common mechanism seen in models, but not mechanisms that oscillate through e.g. a subcritical Hopf bifurcation. The three states observed in the ramp experiments, a steady state and two oscillatory states, may likewise occur in a distributed model given the right type of bifurcation, but we do not know of any distributed model that is capable of accounting for these dynamics.

However, segregated models may be able to explain this multiplicity of oscillatory states since stable, oscillatory solutions to segregated models occur in several modes [19]. Being a budding yeast, \textit{S. cerevisiae} forms two cells of different sizes at division, a larger cell called the mother cell and a smaller cell called a daughter cell. Daughter cells grow to the size characteristic of newly formed mother cells, at which point they are considered as mother cells and can commence a budding cycle. Each oscillatory mode to the age distribution balance for this cell cycle is characterized by two integers, the number of partially synchronized mother cell subpopulations and the number of partially synchronized daughter cell subpopulations. By partially synchronized subpopulations, we mean a cohort of cells that are approximately in the same state and that divide at approximately the same time. The period between
the division times of cohorts of cells equals the period of oscillation of the mode. Cell number balances on the subpopulations provide a unique value for $DP$, the product of dilution rate and the period of oscillation of the mode. However, the value of $DP$ does not differ significantly between modes and an unambiguous identification of a mode through measurements of this product requires more accurate measurements of the dilution rate than was possible in our experiments. Furthermore, deviations from the theoretical value of $DP$ occurs when the cell number balances are altered due to cell death or if age distributions of the partially synchronized cell cohorts overlap. The assumption that cell cycle arrest occurs with some probability $p$ was used by Duboc and Stockar [10] to explain deviation from the theoretical $DP$. They assumed that the observed oscillations were of a mode with 1 mother and 1 daughter cell subpopulation and found the value of $p$ between different experiments varied between 0.53 and 0.93. Of course, this begs the question of why $p$ would vary between experiments? We suggest that the different values of $DP$ may be caused by different modes, or attractors as they would be called in the language of nonlinear mathematics.

It is not known whether the steady state and the oscillatory solutions that are found in segregated models are all stable. Segregated models or population balances are partial, differential-integral equations, coupled with ordinary differential equations, and the mathematical tools for rigorously determining the stability of solutions to these equations are not yet available. Numerical simulations of an age
distribution model (see chapter 3) indicate that the steady and oscillatory states may in fact both be stable under certain operating conditions. Similarly, we cannot know for a fact that the experimentally observed oscillations and steady states are not slow transients or that they are observed at precisely the same operating conditions. This is primarily due to inaccuracy with which the dilution rate is known. A way of detecting transients in the measured signals is by examination of the Fast Fourier Transform of the measurement [5]. Using this technique, we were unable to detect any transients in the signals other than a slight transient adjustment of the period following the onset of the oscillations. It is therefore reasonable to assume that the observed states are stable, barring the effect of wall growth or other slow processes that slowly alter the system and the modeling assumptions.

The segregated mechanism of yeast oscillations explain how the oscillations are stabilized through a feedback between the medium components and the cell cycle control, but do not explain how the oscillations start. Consequently, partial cell cycle synchrony is required in order for a culture to start oscillating. In a batch process, this synchrony usually occurs without intervention as the culture approaches the stationary phase because marked cell size synchronization is associated with the depletion of ethanol [31, 38]. The intent behind the switching experiments was to circumvent this auto-synchrony by switching to continuous operation before auto-synchrony occurs. However, a batch-like growth curve was observed in all switching experiments, indicating that the culture did auto-synchronize, and in all
cases, oscillations began at the end of the batch-like growth curve. The results are in agreement with a segregated mechanism, but can equally well be explained by a distributed model.

The addition of a randomized cell inoculum was able to prevent oscillations from occurring. This is readily explainable in the context of a segregated mechanism of oscillation. Addition of externally grown cells altered the distribution of states of the culture, decreasing the degree of synchrony, such that it ended up in the basin of attraction of the steady state. The number of cells added in these experiments was a small fraction of the total cell number in the fermenters; 5% or less, and it is surprising that such a small perturbation in the distribution of states is sufficient to push the system into the basin of attraction of the steady state.

The results clearly show that the attractor that is observed in a given continuous culture is not just a function of the operating conditions but also depends on the initial conditions or the start up strategy for the culture. From an engineering perspective, it is obviously desirable to know how to reach a given attractor or what the basin of attraction for a given attractor is. Certainly, a culture must be partially synchronized in order to be in the basin of attraction of an oscillatory state. But the concept of “partial synchrony” is disappointingly vague. Perfect synchrony, in which all cells divide simultaneously, is an idealization that cannot be realized in the laboratory and partial synchrony just indicates that the rate of cell division is not constant but is higher at some times than at other times. Any culture that
is not in a steady state is partially synchronized. It is clearly desirable to try and obtain a more rigorous description of the type of synchrony that is required for the oscillations to start. Unfortunately, this is a difficult task. In order to specify the basin of attraction of a given attractor, one must specify all the scalar state variables of the culture, such as medium concentrations and operating parameters, as well as the distribution of states of the culture. Specifying the latter means specifying a function, so the basin of attraction is an object in infinite dimensions. With only one periodic attractor present, one might hope that a synchrony index, several of which have been proposed [32], would suffice to characterize the basin of attraction. But when working with several periodic attractors, corresponding to different distributions of partially synchronized subpopulations, a single synchrony index is an inadequate concept and the problem of characterizing the basins of attraction of solutions to segregated models becomes increasingly difficult.

A practical way of denoting conditions that lead to a given attractor is suggested by the ramp experiments. In these experiments, different final states were attained for different rates of change in dilution rate. It may therefore be possible to specify the conditions that give rise to a desired state by starting the culture in a well defined state, e.g. a steady state under operating conditions that do not support oscillations, and specifying the rate of change in dilution rate that leads to attaining a specified attractor. Ramp changes in other parameters, such as dissolved oxygen, may also prove useful for this purpose.
We suspect that at some operating conditions, the basins of attraction of different attractors have boundaries that are so convoluted that small changes in initial conditions or small disturbances can alter the final state of the culture. This is suggested by the ramp experiments in which differences in the rate of change in dilution rate gave rise to three different final states but is even more strongly suggested by the experiments in which small amounts of RCI were added to the fermenter. Small and convoluted basins of attraction may also explain why these cultures can at times be so difficult and unpredictable to work with. For instance, we have observed that small disturbances, such as a change of medium vessel or adjustments of the silicone tubing of the feed stream, can result in a switch from steady state to an oscillatory state. We have also found that we can detect contamination in a culture by its effect on the dynamics, typically disappearance of oscillations, much sooner than we can detect the contaminant by microscopic observations of samples taken from the reactor. This sensitivity to small disturbances can be annoying but is not present at all operating conditions. Under some conditions, the steady state or the oscillatory state is quite robust. It may be possible to take advantage of the sensitivity of these cultures to small disturbances because it opens up the possibility of using oscillating cultures as sensitive detectors of changes in the environment such as small changes in oxygen pressure in a gas stream or as detectors of toxins or pathogenic organisms.
2.5 Conclusions

Continuous cultures of *S. cerevisiae* were found to possess at least two oscillatory states and one steady state at the same operating conditions. This observation cannot be explained by any currently proposed distributed models and points to a segregated mechanism of these oscillations.

A small perturbation in the distribution of states of the cells in a reactor can cause the reactor to reach a steady state when an oscillatory state is otherwise attained. This suggests that the boundaries of the basins of attraction of different stable states are highly convoluted, at least at some operating conditions, resulting in these cultures becoming sensitive to small disturbances.
Chapter 3

Dynamics Analysis of an Age Distribution Model of Oscillating Yeast Cultures

3.1 Introduction

Bakers' yeast *Saccharomyces cerevisiae* is an important microorganism in many industries including baking, food manufacturing, brewing and genetic engineering. Continuous cultures of *S. cerevisiae* can exhibit autonomous and sustained oscillations under glucose-limited aerobic conditions for a range of operating parameters [1, 9, 8, 31, 34, 35, 36, 37, 38, 48, 49]. The concentrations of both intracellular and extracellular parameters undergo periodic changes and the oscillations are associated with a marked cell cycle synchronization. These oscillations are referred to as cell-cycle dependent [22] as opposed to other types of observed oscillations that do not result in cell cycle synchrony [22, 23, 14, 43]. In most situations, control issues arise due to these oscillations as they adversely affect the stability and productivity of bioreactors [31, 38, 54]. In some situations, however, it might be desirable to induce and stabilize oscillations to increase the production of metabolites that are produced during a certain phase of the cell cycle [54].
Many experimental studies have been conducted to investigate the cause of the autonomous oscillations and its stabilizing mechanisms [1, 9, 8, 31, 34, 35, 36, 48, 49, 22, 6]. Based on these experiments, numerous conceptual and mathematical models were proposed to describe the oscillations [31, 38, 48, 49, 6, 19, 20, 21, 25]. The proposed models can be generally classified into two categories, segregated models in which cell cycle dynamics interact with medium components to cause the oscillations and stabilize the cell cycle synchrony, and distributed, metabolic models in which bifurcation of metabolic kinetics cause the observed oscillations.

Experimental data from our lab (see chapter 2) indicate that different policies of chemostat start up result in different final dynamical states. Two oscillatory states and a steady state were achieved at similar operating condition in three different experiments. These observations disagree with models that oscillate through a Hopf bifurcation and they are not explained by the published distributed models known to us. This leaves segregated mechanisms as possible explanations of these dynamics.

In this chapter, an age distribution model is investigated for its ability to model the observed multiplicity of stable states. The aim of this study is not to obtain a quantitative fit of the model to experimental data, but to determine whether the bifurcation dynamics of the age distribution model can reproduce the experimentally observed dynamics. A complete dynamics analysis of a population balance model is a very challenging task. The models have infinite dimensional state spaces so the basin of attraction of different attractors can be objects in infinite dimensional
spaces and the models include kinetic functions that must be modeled or for which biologically reasonable expressions must be assumed. In this chapter, we investigate the existence of multiple stable states and explore the effect of changing the shape of the transition intensity functions on the dynamics and bifurcation behavior of the model. The model is based on population balance equations (PBE) coupled with a substrate balance. Since analytical solutions of population balance models for microbial growth can only be obtained under very restrictive simplifying assumptions [19, 20, 27], in this work a numerical solution of the model was obtained by the method of orthogonal collocation on finite elements [40, 11]. The model predicts several oscillatory attractors, each of which is characterized by a unique structure of synchronized subpopulations of cells. Preliminary model simulations appear to predict the existence of both a steady state solution and a stable periodic solution for the model which are feasible under the same operating conditions; the attainment of either depends on the initial age distribution. Nevertheless, the stability of the steady state solution is questionable as will be discussed later.

The rest of the chapter is organized in three sections. In section 3.2, model development and the numerical solution method are reviewed. The results of model simulations and bifurcation analysis are discussed in section 3.3. Finally, the conclusions drawn from this investigation are given in section 3.4.
3.2 Model Development and Method of Numerical Solution

3.2.1 Model Development

Although a mathematical description of cell populations has been introduced more than three decades ago by Fredrickson et al. [13, 44], little application is found in biochemical engineering literature. There are two reasons that hindered the full utilization of population balances. First, the fact that the resulting partial integro-differential equations are difficult to solve. Second, application of the theory requires knowledge of three physiological functions that are difficult to determine experimentally [44]. These functions are, the single cell growth rate, the transition rates between different cell compartments, and the partitioning function which describes how a cell property (e.g. mass) of a dividing cell is partitioned among the two newborn cells [44]. In other particulate systems, the transition and partitioning functions are often called the breakage functions [39]. In the light of these facts, the use of age distribution models brings about several advantages that are worth mentioning. Unlike population balance models based on mass which give rise to partial integro-differential equations, models based on age produce partial differential equations. This happens because all newborn cells have age zero and the integral term for the rate of formation of new cells appear as a boundary condition, the renewal equation, rather than in the population balance itself. Moreover, in age distribution models, two of the three physiological functions mentioned above
need not be determined experimentally. First, the single cell growth rate is simply unity and second there is no partitioning function since all cells are born at age zero. The transition functions, however, remain to be estimated in age distribution models. These functions are dependent on both the cell state (e.g. age or mass) and the environmental conditions. For modeling purposes, environmental conditions can be lumped in a single variable such as the substrate concentration. In the current modeling approach, no chemical structure is assumed for either the biophase or the environment, therefore, the model under study is a segregated unstructured model. This type of model is chosen because it is probably the simplest model form that is able to predict periodic behavior of the cell population and its relation to cell cycle synchrony [19, 20].

The age distribution model is based on the following simplified cell cycle of budding yeast. The cycle is characterized by a critical cell mass $m_s$. Cells with mass greater than $m_s$ are called mothers, while cells of smaller mass are called daughters. The point of the cell cycle when a cell reaches $m_s$ is called the “Start” [48, 15, 29]. Cells at the start grow for a period of time, $U_p$, before the emergence of a bud. Budded cells continue growing until the division age is reached. This period is referred to as budded phase period, $B$. At division the bud separates forming a new daughter cell and the original mother cell is back to the “Start” point as a new mother. In budding yeast the division is asymmetric and the newborn daughter cell is born at a smaller mass than the mother. Daughter cells grow until they
reach $m_s$ at which point they join the mother cells population as new mothers. Asymmetric division approaches symmetric division only at maximum growth rate [15, 29]. Mother cells at the "Start" point have age of zero, they grow until they reach the division age, at which point a mother cell divides into a daughter cell and a newborn mother cell both at age zero. Daughter cells grow until they reach the transition age, the point at which they become mothers and their age is reset to zero. This cell cycle is simplified in the sense that there is no distinction between budded and unbudded mother cells. This simplification should not impose a problem unless the environmental conditions become severely poor. Under poor conditions, unbudded mother cells rest in their then-current states and do not bud until the growth conditions improve. On the other hand, budded mother cells are committed to divide, and therefore, they grow for a specific period of time and then divide and rest at their newborn states.

The current model is an enhanced version of the age distribution model originally proposed by Hjortsø and Nielsen [20]. In the former model, both transition and division events occur at discrete ages, referred to as control points, while in the current model both events are modeled by probability functions. The enhancements make the model more biologically plausible since it accounts for individual differences between cells which cause the transition and division to occur over ranges of ages rather than at specific points. Another enhancement of the model was the use of a second order delayed substrate signal instead of using a hard delay to account
for the fact that cell metabolism does not respond instantaneously to environmental changes. An effect similar to having a hard time delay can still be achieved by increasing the order of delay in the filtered signal.

Using the formulation of Fredrickson et al. [13], the PBE for the age distributions of mothers and daughters, $W_m$ and $W_d$, respectively, in a CSTR with dilution rate $D$, are:

\[
\frac{\partial W_m(a,t)}{\partial t} + \frac{\partial W_m(a,t)}{\partial a} = -[D + \Gamma_D(a, S^{(2)})]W_m(a,t) \tag{3.1}
\]

\[
\frac{\partial W_d(a,t)}{\partial t} + \frac{\partial W_d(a,t)}{\partial a} = -[D + \Gamma_T(a, S^{(2)})]W_d(a,t) \tag{3.2}
\]

where $a$ is cell age and $t$ is time. The functions $\Gamma_D(a, S^{(2)})$ and $\Gamma_T(a, S^{(2)})$ are the division and transition intensity functions, respectively. The division intensity function is defined such that $\Gamma_D(a, S^{(2)})dt$ is the probability that a mother cell with age $a$ will divide in the next $dt$ time interval. Similarly, $\Gamma_T(a, S^{(2)})dt$ is the probability that a daughter cell with age $a$ will become a mother in the next $dt$ time interval. $S^{(2)}$ is the second order filtered substrate concentration, also referred to as the effective substrate concentration.

To account for the influx of cells due to cell birth or cell transition, two more equations are needed. These equations are the renewal equations.

\[
W_m(0,t) = W_{m0}(t) = \int_0^\infty \Gamma_D(a, S^{(2)})W_m(a,t)da + \int_0^\infty \Gamma_T(a, S^{(2)})W_d(a,t)da \tag{3.3}
\]

\[
W_d(0,t) = W_{d0}(t) = \int_0^\infty \Gamma_D(a, S^{(2)})W_m(a,t)da \tag{3.4}
\]
The division and transition intensity functions are assumed to have the forms:

\[
\Gamma_{D}(a, S^{(2)}) = \begin{cases} 
0 & a \leq a_{cd}(S^{(2)}) \\
\alpha_{d}(a - a_{cd}(S^{(2)}))^{n_{d}} & a_{cd}(S^{(2)}) < a \leq a_{cd}(S^{(2)}) + \delta_{d} \\
\Gamma_{Dmax} & a > a_{cd}(S^{(2)}) + \delta_{d}
\end{cases}
\]  

(3.5)

\[
\Gamma_{T}(a, S^{(2)}) = \begin{cases} 
0 & a \leq a_{ct}(S^{(2)}) \\
\alpha_{t}(a - a_{ct}(S^{(2)}))^{n_{t}} & a_{ct}(S^{(2)}) < a \leq a_{ct}(S^{(2)}) + \delta_{t} \\
\Gamma_{Tmax} & a > a_{ct}(S^{(2)}) + \delta_{t}
\end{cases}
\]  

(3.6)

where \(a_{cd}(S^{(2)})\) and \(a_{ct}(S^{(2)})\) are the critical age of division and the critical age of transition, respectively, which are the ages beyond which cell division or cell transition commence. \(\Gamma_{Dmax}\) and \(\Gamma_{Tmax}\) are the maxima of the division and transition intensity functions, respectively. \(\alpha_{d}, n_{d}, \delta_{d}, \alpha_{t}, n_{t}\) and \(\delta_{t}\) are constant model parameters. \(\delta_{d}\) and \(\delta_{t}\) are chosen such that the intensity functions are piecewise continuous, giving rise to the expressions \(\delta_{d} = \left(\frac{\Gamma_{Dmax}}{\alpha_{d}}\right)^{\frac{1}{n_{d}}}\) and \(\delta_{t} = \left(\frac{\Gamma_{Tmax}}{\alpha_{t}}\right)^{\frac{1}{n_{t}}}\).

The critical ages of division and transition in this model were assumed to have analogous forms to the ages of division and transition functions in the original model [20]. However, the delayed substrate concentration was replaced by the effective substrate concentration as follows:

\[
a_{cd}(S^{(2)}) = \pi_{0} + \frac{\pi_{1}}{S^{(2)}}
\]  

(3.7)
These model equations were chosen because they are of the simplest possible functional forms that reflect two important physiological phenomena. First, the division age typically increases when substrate concentration decreases, reflecting a lower population growth rate at lower substrate concentrations. Second, at the highest possible growth rates, which typically occur at high substrate concentrations, the transition age becomes so small that the division effectively becomes symmetric [15, 29]. The delayed response is accounted for by the use of the effective substrate concentration instead of the actual concentration. $\pi_0$, $\pi_1$, and $\pi_2$ are constant model parameters.

The substrate balance equation is written as:

$$\frac{dS}{dt} = D(S_f - S) - \frac{1}{\lambda_m(S)} \int_0^\infty W_m(a, t)da - \frac{1}{\lambda_d(S)} \int_0^\infty W_d(a, t)da$$  \hspace{1cm} (3.9)$$

where $S$ is the actual substrate concentration, $S_f$ is the feed substrate concentration and $\lambda_m(S)$ and $\lambda_d(S)$ are the yield coefficients for mothers and daughters, respectively. The integrals appearing in the second and third terms on the right hand side of equation 3.9 are the zeroth moments of the mother and daughter cell age distributions, which by definition are the mother and the daughter cell number
concentrations, respectively. The yield coefficients are assumed to have the form:

\[
\lambda_m(S) = \frac{K_m + S}{\mu_m S}
\]  

(3.10)

\[
\lambda_d(S) = \frac{K_d + S}{\mu_d S}
\]

(3.11)

where \(K_m, \mu_m, K_d\) and \(\mu_d\) are constant parameters. The yield coefficients are the rate of cell age increase divided by the rate of substrate consumption. The rate of cell age increase is unity and the \(\lambda\)'s are therefore the inverse of the rate of substrate consumption by cells of age \(a\). Modeling the rate of substrate consumption by the commonly used Monod rate expression, equations 3.10 and 3.11 are obtained.

The filtered substrate concentrations are written as:

\[
\frac{dS^{(1)}}{dt} = \alpha(S - S^{(1)})
\]

(3.12)

\[
\frac{dS^{(2)}}{dt} = \alpha(S^{(1)} - S^{(2)})
\]

(3.13)

where \(S^{(1)}\) is the first order filtered substrate concentration and \(\alpha\) is the adaptivity constant which determine how rapidly cells respond to environmental changes [47].

The values of the model parameters are given in Table 3.1. Two sets of transition and division intensity functions were used in model simulations and system analysis. These sets of intensity functions differ only in the values of their parameters as shown in Table 3.2. The intensity functions are referred to as moderate and sharp
depending on the size of the age interval over which the function increase from 0 to its maximum. The parameter values were not obtained by a fit to experimental data but were found by trial an error to produce biologically reasonable results.

Table 3.1: Parameters of the Age Distribution Model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$</td>
<td>20.0 g/L</td>
</tr>
<tr>
<td>$K_m$</td>
<td>20.0 g/L</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>2.5 1/hr</td>
</tr>
<tr>
<td>$\mu_d$</td>
<td>4.7e-11 g/hr</td>
</tr>
<tr>
<td>$\mu_m$</td>
<td>4.7e-11 g/hr</td>
</tr>
<tr>
<td>$\pi_0$</td>
<td>1.5 hr</td>
</tr>
<tr>
<td>$\pi_1$</td>
<td>0.5 hr g/L</td>
</tr>
<tr>
<td>$\pi_2$</td>
<td>3.0 hr g/L</td>
</tr>
</tbody>
</table>

Table 3.2: Parameters of the Transition and Division Intensity Functions

<table>
<thead>
<tr>
<th>Moderate Intensity Functions</th>
<th>$\Gamma_{T1}$</th>
<th>$\Gamma_{D1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_T$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$\alpha_T$</td>
<td>300 1/hr$^5$</td>
<td>$\alpha_D$ 300 1/hr$^5$</td>
</tr>
<tr>
<td>$\Gamma_{Tmax}$</td>
<td>20 1/hr</td>
<td>$\Gamma_{Dmax}$ 20 1/hr</td>
</tr>
<tr>
<td>$\delta_T$</td>
<td>0.51 hr</td>
<td>$\delta_D$ 0.51 hr</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sharp Intensity Functions</th>
<th>$\Gamma_{T2}$</th>
<th>$\Gamma_{D2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_T$</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$\alpha_T$</td>
<td>800 1/hr$^4$</td>
<td>$\alpha_D$ 800 1/hr$^4$</td>
</tr>
<tr>
<td>$\Gamma_{Tmax}$</td>
<td>20 1/hr</td>
<td>$\Gamma_{Dmax}$ 20 1/hr</td>
</tr>
<tr>
<td>$\delta_T$</td>
<td>0.29 hr</td>
<td>$\delta_D$ 0.29 hr</td>
</tr>
</tbody>
</table>

3.2.2 Numerical Solution

The current model consists of a coupled set of nonlinear algebraic, ordinary differential, and partial differential equations. An analytical solution of the model is not
possible, and therefore, numerical solution is needed to perform model simulations and bifurcation analysis. Possible candidates of numerical methods are the method of finite differences (MFD) and the method of weighted residuals (MWR) [40, 11]. In general, pure finite differences schemes do not result in satisfactory solutions for PBE models due to various reasons, such as numerical instabilities, low accuracy, and lack of dissipativity, which calls for the use of special forms “hybrid” MFD [30].

The use of the method of weighted residuals for solving mass PBE models was illustrated by Subramanian and Ramkrishna [50]. The method of orthogonal collocation is a variation of MWR. In this method the problem is approximated by coupled, ordinary differential equations for the values of the dependent variable at the collocation points. The method is easy to apply and program for this problem and the solution at any point can be obtained from the values of the dependent variables at the collocation points. The method of orthogonal collocation is also superior to finite differences in terms of stability because it uses the information from all collocation points, instead of just neighboring points, to estimate the derivatives at each point [40]. In this work, the method of orthogonal collocation on finite elements was employed to obtain the numerical solution desired. In orthogonal collocation on finite elements, the domain of the problem is divided into subdomains (called elements) and the method of orthogonal collocation is applied on each subdomain, this variation is particularly useful for problems with sharp variations in the distribution of states [40] such as our problem.
The method of orthogonal collocation on finite elements was found to provide stable and robust solutions of the age distribution model. The method divides the mother and daughter age domains into elements and further discretizes the elements into a number of internal collocation points and two boundary points. From this point on, we will refer to all the internal collocation points and the boundary points simply as collocation points, unless otherwise specified. The partial derivative of $W_m$ or $W_d$ with respect to age at a collocation point in a specific element is approximated by a linear combination of the age distribution values at all the collocation points in that element, except for the lower boundary of the first element in each domain. The elements are constructed such that the lower boundary of the first element starts at age zero, and therefore, at these two boundaries the renewal equations are applied. Consequently, the method approximates the partial differential PBEs by coupled sets of ordinary differential equations (ODEs) for the values of the distribution of states at the collocation points. Integral terms are approximated by Gaussian quadrature [11]. The resulting nonlinear ODEs in vector-matrix notation have the forms:

\[
\frac{d}{dt} W_m = -A_m W_m - D W_m - \Gamma_D W_m - W_m0 A_m0 \tag{3.14}
\]

\[
\frac{d}{dt} W_d = -A_d W_d - D W_d - \Gamma_T W_d - W_d0 A_d0 \tag{3.15}
\]

where $W_m$ and $W_d$ are column vectors of the mother and daughter age distribution values at each collocation point, starting from the second collocation point in each
domain. The first collocation points in the mother and daughter domains are \( a_{m0} = 0 \) and \( a_{d0} = 0 \), respectively. \( \mathbf{A}_m \) and \( \mathbf{A}_d \) are the first derivative weight matrices in the mother and daughter domains, respectively. \( \mathbf{\Gamma}_D \) and \( \mathbf{\Gamma}_T \) are diagonal matrices of the division and transition intensity functions. \( \mathbf{\Gamma}_D \) is defined such that the element \( \mathbf{\Gamma}_D(i,i) \) is \( \Gamma_D(a_{mi}, S^{(2)}) \). \( \mathbf{\Gamma}_T \) is defined in a similar manner in the daughters domain. \( W_{m0} \) and \( W_{d0} \) are the mother and daughter age distribution values at \( a_{m0} \) and \( a_{d0} \), respectively. \( \mathbf{A}_{m0} \) is a column vector that accounts for the contribution of \( W_{m0} \) to the derivatives in the first finite element in the mother domain and \( \mathbf{A}_{d0} \) accounts for the corresponding contribution of \( W_{d0} \) in the first element in the daughter domain.

The renewal equations are written as:

\[
W_{m0}(t) = \sum_{i=1}^{N_m} w_{gm}(a_{mi}) \Gamma_D(a_{mi}, S^{(2)}) W_m(a_{mi}, t) + \sum_{i=1}^{N_d} w_{gd}(a_{di}) \Gamma_T(a_{di}, S^{(2)}) W_d(a_{di}, t) \tag{3.16}
\]

\[
W_{d0}(t) = \sum_{i=1}^{N_m} w_{gm}(a_{mi}) \Gamma_D(a_{mi}, S^{(2)}) W_m(a_{mi}, t) \tag{3.17}
\]

where \( N_m \) and \( N_d \) are the total number of collocation points in the mother and daughter domains, respectively. \( a_{mi} \) and \( a_{di} \) are the ages at collocation point \( i \) in the mother and daughter domains, respectively. \( w_{gm} \) and \( w_{gd} \) are vectors of quadrature weights in the mother and daughter domains, respectively. The substrate balance
is written as:

\[ \frac{dS}{dt} = D(S_f - S) - \frac{1}{\lambda_m(S)} \sum_{i=0}^{N_m} w_m(a_{mi}) W_m(a_{mi}, t) - \frac{1}{\lambda_d(S)} \sum_{i=0}^{N_d} w_d(a_{di}) W_d(a_{di}, t) \]

(3.18)

The rest of the model equations are unchanged.

Model Simulations

Model simulations were performed using MATLAB. The model equations were solved using ODE15s, a stiff ODE solver. An analytical expression of the Jacobian matrix was provided to the solver. In previous work, we have used a mesh of fixed equal sized elements to solve a mass PBE model of budding yeast using the method of orthogonal collocation on finite elements [54]. In the current study, dynamic meshes of 15 elements in the mother domain and 18 elements in the daughter domain were used. Each element had 3 internal collocation points obtained as the roots of the appropriate Jacobi polynomials [40]. The total number of collocation points in the mother domain is \( N_m = 61 \) and in the daughter domain is \( N_d = 73 \).

The state vector of the resulting ODE model consists of the cell age distribution at each collocation point, as well as, the substrate and the first and second order filtered substrates. In the placement of the elements, each domain is divided into three subdomains. The first, subdomain 1, extends from \( a = 0 \) to \( a = (a_c - 0.2) \) or \( a = (a_c - 0.3) \) hr, where \( a_c \) is the critical age of either transition or division. Subdomain 2 starts at the end of subdomain 1 and extends for 1.4 hours. Finally,
subdomain 3 starts at the end of subdomain 2 and extends for 1 hr. The distribution of states changes most rapidly in subdomain 2 as the intensity functions sharply increase from zero to their maxima, therefore, at least one third of the elements is stacked in subdomain 2. All the transition and division events are expected to take place in subdomain 2, however, 1 element is placed in subdomain 3 to take care of any remaining cells as an extra precaution. The rest of the elements are placed in subdomain 1. If the element size in subdomain 1 is smaller than that in subdomain 2, some elements are transferred to subdomain 2, where they are needed most. The positioning of the elements is not fixed, the critical ages are checked every one virtual hour, and if the value of either critical age change by more than 0.1 hr, the elements are re-positioned. The values at the new collocation points were obtained using Lagrange interpolating polynomials in each element [7]. If the new age domain was larger than the old one, the age distribution would be set to 0 at the new age collocation points that exceed the range of the old domain.

Bifurcation Analysis

The bifurcation analysis was done using code provided by Dr. Kevrekidis, Princeton. The shooting method was used to perform the continuation steps in the bifurcation analysis. The continuation code was written in FORTRAN and it uses the stiff ODE solver, ODESSA, with explicit simultaneous sensitivity analysis to solve the ODE model. An analytical expression of the Jacobian matrix was also provided to the ODE solver. For every oscillatory attractor, the code requires an initial
age distribution and a guess of the period, which were obtained from MATLAB simulation results. The same number of elements and internal collocation points used in model simulations was used again here. For the bifurcation analysis, however, simpler fixed meshes were utilized. The end of each age domain was fixed and the elements were distributed such that the last hour of each domain was a single element, and the remaining elements were equally divided in the rest of the domain.

3.3 Results and Discussion

3.3.1 Simulation Results

Oscillatory solutions occur less readily in the current model than in the simplified control point model [20] due to the dispersive effect of the transition and division intensity functions. In this chapter, two sets of intensity functions were investigated, the parameters of which are given in Table 3.2. The sharpness of the functions are related to the rate at which the function increase from 0 to its maximum value. Since the maxima of both intensity functions were the same, the sharpness of these functions are directly related to the parameters $\delta_T$ and $\delta_D$. The smaller the values of $\delta_T$ and $\delta_D$, the sharper the transition and division intensity functions are, respectively. Stable oscillatory solutions were still possible even in the case of moderate transition and division intensity functions $\Gamma_{T_1}$ and $\Gamma_{D_1}$, however, further increase in $\delta_T$ and $\delta_D$ led to the loss of periodic solutions.
Using the moderate intensity functions $\Gamma_T$ and $\Gamma_D$, two periodic attractors were found at feed substrate concentration $S_f = 30 \text{ g/L}$. By a periodic or oscillatory attractor we mean the set of oscillatory solutions which are characterized by a specific subpopulations structure in the mother and daughter domains. For example, the N:M attractor is the set of oscillatory solutions with N subpopulations in the mothers domain, as indicated by N local maxima in the mother age distribution and M subpopulations in the daughters domain [19, 20]. Figure 3.1 shows typical age distributions encountered in the 1:2 and 1:3 attractors. The periodic attractors were found in two separate regions of dilution rate $(D)$ values. Typical oscillation patterns in the substrate concentration $(S)$ and cell number concentrations $(N_c)$ for the 1:2 attractor at $D = 0.15 \text{ hr}^{-1}$ are shown in figure 3.1. The figure also shows the corresponding oscillations at $D = 0.095 \text{ hr}^{-1}$, which belong to the 1:3 attractor. Naturally, the mother and daughter age distributions associated with the oscillatory patterns shown in figure 3.1 are time varying, however, a representative snap shot of the age distributions in each case is shown below their respective patterns.

The range of dilution rate supporting each of the two oscillatory attractors were estimated using MATLAB simulations. The procedure is summarized as follows: first a stable oscillatory solution needs to be determined at some value of dilution rate. Then this value of dilution rate is changed slowly to a new level. Afterwards, the simulation is continued at the new $D$ until the oscillations either stabilize or disappear. Despite the good estimates obtained by this procedure, the method was
Figure 3.1: Typical oscillation patterns associated with the 1:2 attractor (a) and 1:3 attractor (b). At $S_f = 30$ g/L and $D = 0.15 \text{ hr}^{-1}$ a periodic solution that belongs to the 1:2 attractor is obtained. Oscillations are observed in all system parameters such as cell number concentration (a-1) and substrate concentration (a-2). The 1:2 attractor is characterized by one subpopulation of mothers (a-3) and two subpopulations of daughters (a-4). At $D = 0.095 \text{ hr}^{-1}$ a periodic solution that belongs to the 1:3 attractor is obtained. Oscillations are also observed in all system parameters such as cell number concentration (b-1) and substrate concentration (b-2). The 1:3 attractor is characterized by one subpopulation of mothers (b-3) and three subpopulations of daughters (b-4).
inefficient. First, long execution times were needed to assure the stability of the obtained oscillations. Second, near the end of the dilution rate range, within which oscillations are observed, the execution times required increase dramatically because the periodic solutions take much longer times to either stabilize or die out. Finally, only few discrete points can be obtained in a reasonable time frame, which imposes a limit on the resolution of the results and the ability to produce good interpolations. Therefore, a faster, more accurate and more reliable procedure was desired. The use of a continuation code utilizing the shooting method to determine the whole range of limit cycles in a given attractor was found to be a more practical procedure. The code requires an initial age distribution and a reasonably “good” guess of the period, which were readily available from MATLAB simulations. The results obtained from the continuation code were in good agreement with those obtained from MATLAB simulations. The maximum and minimum values of the periodic solutions of $S$ and $N_c$ obtained from MATLAB simulations at discrete values of $D$ are shown as $\bullet$ in figure 3.2 for the 1:2 and 1:3 attractors. The dashed lines in figure 3.2 represent the results obtained from the continuation code and the solid line represents the steady state profile. At steady state the equations of the PBE model become a set of nonlinear algebraic expression coupled with two ODEs. With the type of expressions used in this analysis an analytical solution for the steady state profile, which is shown in figure 3.2, was possible. It was found that all the system variables oscillate around their steady state values.
Figure 3.2: Bifurcation diagram at $S_f = 30$ g/L, obtained by employing moderate intensity functions $\Gamma_{T_1}$ and $\Gamma_{D_1}$. Bifurcations are shown in terms of cell number concentration (a) and substrate concentration (b). The diagram shows maximum and minimum cell number and substrate concentration during the oscillations obtained from MATLAB (●), maximum and minimum cell number and substrate concentration during the oscillations obtained from the continuation code (- -) and the steady state profiles (—).

When the simulations were performed using the sharp intensity functions $\Gamma_{T_2}$ and $\Gamma_{D_2}$, the regions of $D$ supporting the 1:2 and 1:3 attractors expanded and shifted towards higher values of $D$. The ranges of $D$ supporting the 1:2 attractor under the moderate and sharp intensity function conditions are compared in figure 3.3. In addition to the original two attractors a third attractor was found, the 1:1 attractor. The bifurcation diagram representing the 1:1 and 1:2 attractors is shown in figure 3.4. The discovery of the 1:1 attractor was not surprising, it has the simplest
structure of the three attractors and intuitively it was expected to be the easiest attractor to locate. However, all our attempts to locate the 1:1 attractor using $\Gamma_{T1}$ and $\Gamma_{D1}$ failed. In fact, the 1:1 attractor may not exist when moderate transition intensity functions are used. The locus of the 1:1 attractor was found at higher dilution rates than the other two attractors and at higher values of dilution rate the average substrate and effective substrate concentrations are also higher. This results in smaller averages for the critical ages of division and transition, $a_{ct}$ and $a_{ct}$, than at lower values of the dilution rate. The range of ages over which the mother and daughter distribution functions, $W_m$ and $W_d$, are significantly different from zero is directly related to $a_{ct}$ and $a_{ct}$ and both ranges are smaller at higher dilution rates than at lower dilution rates. Consequently, the intensity functions become relatively more dispersive at higher dilution rates, eliminating stable oscillatory solutions. In other words, the size of $\delta_T$ and $\delta_D$ is only a relative measure of the sharpness of $\Gamma_T$ and $\Gamma_D$ and hence the “sharpness” of $\Gamma_T$ and $\Gamma_D$ with fixed $\delta_T$ and $\delta_D$ is not the same in all attractors. The sharpness of the intensity functions should be thought of as the ratio of $\delta_T$ and $\delta_D$ to the age domains of $W_d$ and $W_m$, respectively. Of course, this reasoning is only valid when the transition intensity functions, equations 3.5 and 3.6, are not functions of the dilution rate, substrate or effective substrate concentrations.

The effect of the feed substrate concentration ($S_f$) on the range of $D$ supporting the oscillatory attractors was investigated. Three levels of $S_f$ were studied, 15, 30
Figure 3.3: The effect of the "sharpness" of intensity functions on the range of dilution rates supporting the 1:2 attractor. The curves show the maximum and minimum values of the substrate concentration during the oscillations under moderate intensity functions ($\Gamma_{T1}$ and $\Gamma_{C}$) (---) and sharp intensity functions ($\Gamma_{T2}$ and $\Gamma_{D2}$) (---) parameter values.

and 60 g/L. It was found that the ranges of $D$ at which the attractors exist decrease with increasing $S_f$. As a matter of fact, we were unable to locate the 1:3 attractor at $S_f = 60$ g/L. Figure 3.5 shows the effect of increasing $S_f$ on the domain of the 1:2 attractor in terms of $D$. The upper bifurcation point of $D$ is almost the same in all three cases, however, periodic dynamics start to appear at lower values of $D$ at lower $S_f$. It should be noted though that increasing $S_f$ does not affect the steady state value of $S$ and neither does it affect the average substrate concentration for an oscillating culture. Therefore, the decrease in the domain of the 1:2 attractor is not due to any change in the values of the critical ages or the relative sharpness of the intensity functions. The effect of increasing the feed substrate concentration is directly translated into an increase in the steady state value of the cell number.
Figure 3.4: Bifurcation diagram at $S_f = 30\text{ g/L}$, obtained by employing sharp intensity functions $\Gamma_{T1}$ and $\Gamma_{D1}$. The diagram represents the 1:1 and 1:2 attractors in terms of cell number concentration (a) and substrate concentration (b). The diagram shows maximum and minimum cell number and substrate concentration during the oscillations obtained from the continuation code (---) and the steady state profiles (—).

concentration, or the average cell number concentration for an oscillating culture. In this case, the reduction of the 1:2 attractor domain is attributed solely to the change in operating conditions.

The results obtained from model simulation indicate the existence of multiple oscillatory attractors, which has been confirmed experimentally. In our experiments, however, the different attractors have been observed at similar operating conditions, while in simulations the ranges of $D$ supporting the different attractors do not in-
Figure 3.5: The effect of feed substrate concentration, $S_f$, on the range of dilution rate supporting the 1:2 attractor. The curves show the maximum and minimum values of the substrate concentration during the oscillations at $S_f = 15$ g/L (—), 30 g/L (- -) and 60 g/L (···). 

tersect. However, it has been demonstrated that the domains of the oscillatory attractors are strong functions of both model parameters and operating conditions. Therefore, reproduction of experimental observations is conceivable given sufficient time and computing power to adjust the model parameters and transition probabilities properly.

### 3.3.2 Bifurcation Analysis Results

In our experimental effort to investigate the bifurcation dynamics of continuous yeast cultures, an experiment was performed in which the periodic dynamics of an oscillating chemostat culture were quenched by slowly reducing the dilution rate to a value that did not support oscillations. The chemostat was run for two days at the new conditions then the dilution rate was increased slowly, so as to keep the...
system at a quasi-steady state, to its original value. The non-oscillatory state was preserved all through the ramp and it was sustained at the original dilution rate for almost three more days. A simulation of the experiment was performed and the results obtained were qualitatively very similar to those obtained experimentally. Both experimental data in terms of the exhaust CO$_2$ and the corresponding model simulations in terms of substrate concentration are shown in figure 3.6.

Figure 3.6: Bifurcation dynamics of budding yeast investigated experimentally (a) indicate that an oscillatory state and a steady state can be achieved at the same operating conditions. Model simulations (b) predict the same bifurcation mechanisms. In the top figure, the actual response of CO$_2$ concentration in the exit gas stream (—) to set point changes in the dilution rate (---) is shown. In the bottom figure, the corresponding simulated response of substrate concentration (—) to changes in dilutions rate (---) is shown.
From nonlinear system dynamics viewpoint, since the model predicts the existence of a stable limit cycle that encircles a stable steady state, one expects an unstable limit cycle somewhere in between [24]. A powerful feature of the continuation code is its ability to find both stable and unstable limit cycles. At the end points of the dilution rate region within which oscillatory attractors prevail, the stable limit cycle shrinks and approaches the steady state. If an unstable limit cycle exists between the stable limit cycle and the stable steady state, the continuation code is expected to find it near those points. In all the attractors studied, the attempts of finding such unstable limit cycles were unsuccessful. However, when the amplitude of the oscillations diminishes as the limit cycles approach the steady state, it becomes increasingly tedious and time consuming to perform the analysis. At these points, smaller step sizes should be specified among other adjustments to the code parameters. The stable limit cycle keeps getting smaller until it collapses at the steady state. The failure of the continuation code in finding the unstable limit cycles raises doubt about the original assumption that the steady state is indeed stable.

Model simulations by MATLAB show that integrating the model equations starting with the analytical steady state distribution as the initial condition, the system remains at steady state for hundreds of virtual hours. Further simulations indicated that the system still arrives to the steady state solution even if small disturbances were introduced to the initial steady state distribution. These simulations indicate
the stability of the steady state, and the existence of at least a small region of attraction for this state. However, the eigenvalues of the Jacobian matrix tell another story. When the eigenvalues of the Jacobian matrix were evaluated at $S_f = 30 \text{ g/L}$, $D = 0.14 \text{ hr}^{-1}$ and the corresponding steady state distribution, a pair of the eigenvalues was found in the right half plane. The pair was very close to the imaginary axis and the value of the real part, 0.0090, was very small compared to the real part of smallest negative eigenvalue, -55.0729. The condition number of the Jacobian matrix was also very large, $1.565 \times 10^{27}$, indicating that the Jacobian matrix was ill conditioned. In order to investigate whether the discretization process has an effect on the position of the pair of eigenvalues with positive real part or not we have further discretized the system, this time using 22 elements in the mothers domain and 25 elements in the daughters domain with 3 internal collocation points in each element. Once again, one pair of positive eigenvalues was found with a very small real part of 0.0095 compared to the real part of the smallest eigenvalue of -75.2900. Once again, the Jacobian matrix was found to be ill conditioned. Considering the small magnitude of the positive eigenvalues, the ill conditioning of the Jacobian matrices and the error involved in determining their eigenvalues, one can only have limited confidence in the Jacobian matrix analysis. The pair of positive eigenvalues might actually lie on the imaginary axis, in which case, the stability of the system cannot be inferred from the Jacobian matrix but requires analysis of the higher order terms in the Taylor expansions of the ODE right hand sides. Finally, the ODE integrator...
used in the continuation code, ODESSA, was used to integrate the model equations starting with the steady state distribution as the initial condition. Again the system remained at steady state for hundreds of virtual hours. However, there was a significant difference between the solutions obtained using the MATLAB ODE solver, ODE15s, and ODESSA solver. By magnifying the observed steady state signals for substrate concentration, MATLAB simulations reveal a small converging oscillatory signal, while simulations using ODESSA reveal a small diverging oscillatory signal as shown in figure 3.7. The solutions obtained using the ODESSA solver, therefore, indicates that the steady state is not stable. The observations above, although not conclusive, raise reasonable doubt about the stability of the steady state.

Assuming that the unstable limit cycle indeed does not exist as the results of the continuation code imply, then the steady state must become unstable as the oscillations emerge. In most of the attractors studied, the stable limit cycles branch from the steady state and collapse back at the steady state which is a characteristic of supercritical Hopf bifurcation [28]. Only the 1:3 attractor at $S_f = 30 \text{ g/L}$ and the 1:2 attractor at $S_f = 60 \text{ g/L}$ obtained under moderate intensity functions parameters, show a Hopf bifurcation that is subcritical at one end and supercritical at the other end [28]. Even at the end that shows subcritical Hopf bifurcation, the stable limit cycle becomes very small before it branches to an unstable limit cycle that persists for a very small range of the bifurcation parameter before it collapses back at the
Figure 3.7: Results of model simulations performed at the operating conditions $S_f = 30 \text{ g/L}$ and $D = 0.14 \text{ hr}^{-1}$ and starting with the corresponding steady state distribution as the initial condition. Model equations were integrated using MATLAB stiff ODE solver, ODE15s (a) and the continuation code ODE solver, ODESSA (b). The simulations show that the steady state can be observed for hundreds of virtual hours (note the range on the substrate concentration axis), however, simulations performed using ODE15s predict a stable steady state, while simulations performed using ODESSA predict an unstable steady state.

steady state. In all cases, however, the bifurcation mechanism observed was a Hopf bifurcation.

Our results raise three questions: (i) Is the steady state predicted by the model stable or not? (ii) Is the steady state observed experimentally stable or not? (iii) Is it justified to eliminate models just on the basis of their bifurcation mechanisms? More specifically, is it justified to eliminate all models that bifurcate through a Hopf bifurcation mechanism? The answer to the first question is we do not know. Different numerical methods give different and conflicting answers about the sta-
bility of the steady state. However, the important common fact revealed by all the methods was that the system has very slow dynamics and whether the steady state is stable or not, very little change is observed in the steady state conditions even for extended periods of time. This does raise the possibility that the experimentally observed steady states are in fact not stable, but are bifurcating to an oscillatory state, extremely slowly. However, this conclusion is contrary to previous observations that oscillations appear or disappear very rapidly, based on environmental conditions [35]. In chapter 2, it was demonstrated that the actual system is also characterized by slow transients. For instance, FFT analysis revealed that the transients in the oscillatory state take almost two days to die out. Therefore the currently observed non-oscillatory state which persisted for several days may be a true stable steady state or just an extended transient. More experimental work is required to investigate the stability of this state. Finally, the bifurcation mechanism underlying the emergence of oscillations in a certain model does not reveal the whole picture. Knowing that a model bifurcates through a Hopf bifurcation for example does not reveal how many oscillatory solutions are possible, or how rapidly the steady state bifurcates to the oscillatory state. Therefore, more information about model bifurcation than just its bifurcation mechanism may be needed in order to validate or discard the model. Performing a detailed bifurcation analysis is a very useful tool in model discrimination. However, care should be taken not to
generalize the conclusions about the validity of a specific model based solely on its bifurcation mechanism.

3.4 Conclusions

An age distribution PBE model was proposed as an explanation of the autonomous oscillations in budding yeast. The model was solved numerically by the method of orthogonal collocation on finite elements. The model predicts the existence of multiple oscillatory attractors, an observation that is experimentally validated. The range of dilution rates supporting the attractors depend on values of model parameters, the proposed mathematical expressions of different biological functions and the operating conditions. The model also predicts the existence of a stable oscillatory state and a prolonged steady state at the same operating conditions. These predictions are in good agreement with experimental observations. There is conflicting evidence about the stability of the steady state, however. The system dynamics are very slow and the apparent steady state can persist for extended periods of time. A detailed bifurcation analysis can be a useful tool for model discrimination, although care should be taken in drawing conclusions about the validity of models based solely on their bifurcation mechanism.
Chapter 4

Dynamics Analysis of a Hybrid Mass-Age Distribution Model of Oscillating Yeast Cultures

4.1 Introduction

Chemostat cultures of bakers' yeast, *Saccharomyces cerevisiae*, can exhibit autonomous and sustained oscillations in many extracellular and intracellular parameters under glucose limited aerobic conditions [1, 4, 8, 9, 31, 34, 35, 36, 37, 38, 48, 49]. The mechanism that causes these oscillations is contentious and both distributed, metabolic models and segregated models were proposed in literature as explanations of the autonomous oscillations [8, 9, 21, 23, 31, 34, 35, 36, 43]. In chapter 2 of this dissertation, it was demonstrated that the continuous cultures of *S. cerevisiae* exhibit rich dynamics. For instance, starting at different initial conditions, two oscillatory states and an extended non-oscillatory state were achieved at the same set of operating conditions. These dynamics have segregated characteristics and cannot be explained by distributed models.

An age distribution model was proposed in chapter 3. Bifurcation analysis of the model showed its capability of predicting multiple oscillatory attractors. These attractors, however, were located at separate regions of dilution rate, which was
investigated as the bifurcation parameter. The model also predicted the existence of a steady state at the same operating conditions that supports stable oscillations. The stability of this steady state was not resolved, though. While model simulations showed that the steady state is stable, the bifurcation analysis suggested that it is not. In any case, model analysis indicated that the system dynamics are very slow and even if the steady state is indeed unstable, it can prevail for an extended periods of time before oscillations of noticeable amplitude can be observed. Therefore, for all practical purposes, this steady state cannot be distinguished from the experimentally observed non-oscillatory state.

The age distribution model was based on a simplified budding yeast cell cycle. Therefore, it is reasonable to assume that the validity of the model is limited by the validity of the assumptions upon which it was based. A schematic diagram of *S. cerevisiae* cell cycle is shown in figure 4.1. The cell cycle is characterized by a critical cell mass, $m_s$. Cells with mass greater than $m_s$ are called mothers, while cells with less mass are called daughters. The point of the cell cycle when a cell reaches $m_s$ is called the "Start" [15, 29, 48]. Cells at the "Start" grow for a period of time during which they gain sufficient mass to reach another critical mass known as the transition mass, $m_t$. The attainment of this mass is a necessary condition for the commencement of budding. A budded cell continues growing with most of the increase in mass taking place in the bud for a fixed period of time known as the budded phase period. At the end of this period the third critical point in
the cell cycle is reached, namely, the critical age of division, $a_d$. At division, the bud separates, forming a new daughter cell and the original mother cell is back to the “Start” point as a new mother. The division in *S. cerevisiae* is asymmetric and daughters are produced at smaller mass, $m_0$, than mothers are. Therefore, daughters have longer generation time than that of the mothers. Only at maximum growth rates, does asymmetric division approach symmetric division [15, 17, 18, 29].

![Cell cycle diagram](image)

**Figure 4.1: The Cell Cycle of *S. cerevisiae***

The age distribution model was developed based on a simplified cell cycle which was controlled by two critical control points, the age of transition, $a_t$, and the age of division, $a_d$. The age of transition is defined as the age at which daughter cells are transformed into mothers, and thus it is associated with the mass at the “Start” as shown in figure 4.1. In this simplified cell cycle, there is no critical point in terms of age that is associated with the transition mass. Therefore, in the age distribution model there is no distinction between unbudded and budded mother cells. Under
good nutritional conditions, the growth of unbudded cells to the transition mass and
the subsequent budding is not hindered in any way, and thus the distinction between
unbudded and budded cells is not very important. At poor nutritional conditions,
however, the single cell growth rate drops sharply, and unbudded cells do not start
a new budding cycle unless the environmental conditions improve. Budded cells,
on the other hand, are committed to divide. Therefore, at very poor nutritional
conditions, budded cell need to utilize their storage proteins in order to continue
the budding phase period and eventually divide at $a_d$ forming smaller daughter
cells compared to those formed in rich environment. Indeed, this is the reason why
budded yeast batch cultures auto-synchronize as substrates deplete at the end of
the batch growth. This behavior cannot be captured by the age distribution model.
In chapter 3, it was assumed that both the critical $a_i$ and $a_d$ increase indefinitely as
the substrate vanishes. As a result, neither daughter nor mother cells bud or divide.
This contradicts with the actual behavior of budded mother cells, however, which
must divide. Hence the age distribution model is not suited for the description of
situations where yeast cultures are starved.

In this chapter, an alternative population balance model is proposed. This model
accounts for the cell mass distribution of unbudded cells and the age distribution of
budded cells, consequently, it will be referred to as the hybrid mass-age distribution
model. In this model, all three critical points of the cell cycle are considered. It was
found experimentally that the transition mass does not vary much under different
growth conditions [15]. Therefore, for practical purposes, the mass of transition was assumed constant in this model and all cell transitions occur at the transition mass. Under this assumption, all budded cells at the same age will have the same mass; otherwise, budded cells at the same age will have a distribution of masses. If budded cells have distribution of masses at every age, a two dimensional problem would arise in the budded cells domain and the solution of such a problem is far more involved than the one introduced here. To make the model biologically reasonable, it was assumed that the division event is governed by a division intensity function, \( \Gamma_D \), while the distribution of the newborn cells is governed by a birth probability function, \( P \). It is expected that this model will retain the most important features of the age distribution model and at the same time it is expected to be efficient in handling situations where culture starvation is involved. The model equations were solved using the method of orthogonal collocation on finite elements [11, 40].

The objective of this chapter is to investigate the capability of the hybrid mass-age model in predicting the batch growth dynamics including the bimodal mass distribution of the late batch culture. It is also desired to investigate if the current model can predict multiple oscillatory states and the possible existence of an oscillatory state and a steady state at the same operating conditions.

The rest of the chapter is organized in five sections. In section 4.2, the development of the model equations and their simplifying assumptions are discussed. The numerical solution method and the resultant system of ordinary differential equa-
tions are reviewed in section 4.3. The parameters used in model simulations, the solution procedure, detailed derivation of the elements of the Jacobian matrix as well as a complete analysis of the steady state solution are presented in section 4.4. In section 4.5, the most important results and features of the model are discussed. Finally, the conclusions drawn from this investigation are given in section 4.6.

4.2 Model Development

The population balance equations describing the mass distribution of unbudded cells, $W_u$, and the age distribution of budded cells, $W_b$, are:

\[
\frac{\partial W_u(m, t)}{\partial t} + \frac{\partial r(S, S_e)W_u(m, t)}{\partial m} = -D W_u(m, t) + 2 \int_0^\infty P(m, a)\Gamma(a, S_e)W_b(a, t)da
\]

\[
\frac{\partial W_b(a, t)}{\partial t} + \frac{\partial W_b(a, t)}{\partial a} = -(D + \Gamma(a, S_e))W_b(a, t)
\]

(4.1)

(4.2)

where $m$ is cell mass, $a$ is cell age and $t$ is time. The function $r(S, S_e)$ is the single cell growth rate. The function $P(m, a)$ is the birth probability function. The function $P(m, a)$ is defined such that $P(m, a)dm$ is the probability that a newborn cell will have a mass between $m$ and $m + dm$ as a result of the division of a cell of mass $m_b(a)$ where $m_b(a)$ is the mass of a budded cell at age $a$. The function $\Gamma(a, S_e)$ is the division intensity function. The division intensity function is defined such that $\Gamma(a, S_e)dt$ is the probability that a mother cell with age $a$ will divide in the next $dt$
time interval. \( S \) and \( S_e \) are the substrate concentration and the second order filtered substrate concentration, also referred to as the effective substrate.

The boundary conditions for the above two equations arise from the assumptions that there are no cells with mass zero and the flux into the budded cells region at age zero equals the flux out of the unbudded cells region at the transition mass, \( m_t \).

\[
W_u(0, t) = 0 \quad (4.3)
\]

\[
W_b(0, t) \frac{da}{dt} = W_u(m_t, t) \frac{dm}{dt} \quad (4.4)
\]

However, \( \frac{da}{dt} \) is by definition unity and \( \frac{dm}{dt} \) is nothing but the single cell growth rate, \( \gamma(S, S_e) \). Therefore, equation 4.4 becomes:

\[
W_b(0, t) = \gamma(S, S_e)W_u(m_t, t) \quad (4.5)
\]

The mass of a budded cell at age zero is by definition the mass of transition which results in one more condition to be satisfied.

\[
m_b(0) = m_t \quad (4.6)
\]

The single cell growth rate was modeled by a Monod expression. In order to account for the delay between the environmental conditions and cell response, the effective substrate was used in the Monod expression instead of the actual substrate.
concentration. A correction factor was also included to account for the fact that at very low substrate concentration the single cell growth rate diminish regardless of the value of the filtered substrate.

\[
\tau(S, S_e) = \frac{\mu S_e}{(K_1 + S_e)} F(S) \tag{4.7}
\]

where \(\mu\) is the maximum single cell growth rate, \(K_1\) is constant model parameter and \(F(S)\) is a correction factor, \(F(S) = \frac{S}{(K_2 + S)}\). The division intensity function is assumed to have the form:

\[
\Gamma(a, S_e) = \begin{cases} 
0 & a \leq a_{cd}(S_e) \\
\alpha_d(a - a_{cd}(S_e))^{n_d} & a_{cd}(S_e) < a \leq a_{cd}(S_e) + \delta_d \\
\Gamma_{\text{max}} & a > a_{cd}(S_e) + \delta_d 
\end{cases} \tag{4.8}
\]

where \(a_{cd}(S_e)\) is the critical age of division, which is the age beyond which cell division commences. \(\Gamma_{\text{max}}\) is the maximum value of the division intensity function. \(\alpha_d\), \(n_d\) and \(\delta_d\) are constant model parameters. \(\delta_d\) is chosen such that the intensity function is piecewise continuous, giving rise to the expression \(\delta_d = \left(\frac{\Gamma_{\text{max}}}{a_d}\right)^{\frac{1}{n_d}}\).

The critical age of division in this model was assumed to have an analogous form to the age of division function proposed by Hjortsø and Nielsen (1995) at high substrate concentration [20]. In their model, Hjortsø and Nielsen, assumed that at low substrate concentration, the age of division becomes infinitely large. This argument was justified by the observation that unbudded mother cells do not bud
at poor nutritional conditions and consequently never divide. This is one of the weaknesses of that model since it does not account for cells which have already budded before the depletion of substrate. In reality, budded cells are committed to divide and the budded period was found to be constant over a wide range of operating condition. Only at very low substrate concentration does the budded period increase. The above facts were incorporated in the expression used for the critical age of division as a function of effective substrate:

\[
a_{cd}(S_e) = \begin{cases} 
\pi_0 + \frac{\pi_1}{S_e} & \text{if } S_e > S_{\text{min}} \\
\pi_2 & \text{if } S_e \leq S_{\text{min}}
\end{cases}
\] (4.9)

where \(\pi_0, \pi_1, \pi_2\) and \(S_{\text{min}}\) are constant model parameters. Once again, the model parameters were chosen such that the division intensity function is piecewise continuous. Therefore, \(\pi_2 = \pi_0 + \frac{\pi_1}{S_{\text{min}}}\). The birth probability function comprised of two Gaussian peaks centered at the masses of the newborn mother and the newborn daughter as follows:

\[
P(m, a) = \alpha(\exp(-\beta(m_a - m)^2) + \exp(-\beta((m_b(a) - m_a) - m)^2))
\] (4.10)

where \(\alpha\) and \(\beta\) are constant model parameters. At a given time \(t\), the mass of a budded cell of age \(a\) can be found by integrating the single cell growth rate from
time \( t - a \) to \( t \).

\[
\frac{dm_b}{dt} = \frac{dm}{dt} = r(S, S_e)
\]

or

\[
m_b(a) = m_t + \int_{t-a}^{t} r(S, S_e) dt
\]

The birth probability function, \( P(m, a) \), must also satisfy the condition:

\[
\int_0^{m_b(a)} P(m, a) dm = 1
\]

(4.12)

The substrate balance is given by:

\[
\frac{dS}{dt} = D(S_f - S) - \int_0^{m} \frac{1}{\lambda_1} r(S, S_e) W_u(m, t) dm - \int_0^{\infty} \frac{1}{\lambda_2(S, S_e)} W_b(a, t) da
\]

(4.13)

where \( \lambda_1 \) and \( \lambda_2 \) are the yield coefficients. From a physical point of view, the yield coefficient \( \lambda_1 \) can be thought of simply as a constant stoichiometric coefficient that relates the increase of cell mass to the consumption of substrate. On the other hand, the yield coefficient, \( \lambda_2 \), is the rate of cell age increase divided by the rate of substrate consumption. The rate of cell age increase is unity and, therefore, \( \lambda_2 \) is the inverse of the rate of substrate consumption by budded cells of age \( a \). There is a principal difference between the yield coefficients in the mass and age domains. In the mass domain as the environmental conditions become poor the single cell growth rate vanishes and the cells do not grow (in mass) any more. This fact makes it a reasonable assumption to use a constant yield coefficient. However, in the
age domain cells grow with a growth rate of unity regardless of the environmental conditions resulting in a dependency of the yield coefficient on the environmental conditions. It was assumed that $\lambda_2$ satisfies the following expression.

$$\lambda_2(S, S_e) = \frac{\lambda_1}{\tau(S, S_e)}$$  (4.14)

The filtered substrate concentrations are written as:

$$\frac{dS^{(1)}}{dt} = \alpha_a(S - S^{(1)})$$  (4.15)

$$\frac{dS^{(2)}}{dt} = \alpha_a(S^{(1)} - S^{(2)})$$  (4.16)

where $S^{(1)}$ and $S^{(2)}$ are the first and second order filtered substrate concentration and $\alpha_a$ is the adaptivity constant which determine how rapidly cells respond to environmental changes [47]. Note that $S_e = S^{(2)}$.

4.3 Numerical Solution

The method of orthogonal collocation on finite elements was found to provide stable and robust solutions of the mass-age distribution model. The method divides the unbudded cells mass domain and the budded cells age domain into elements and further discretize each element into a number of internal collocation points and two boundary points. From this point on, we will refer to all the internal collocation points and the boundary points simply as collocation points, unless otherwise speci-
fied. The partial derivative of $W_u$ and $W_b$ with respect to mass and age, respectively, at a collocation point in a specific element is approximated by a linear combination of the distribution values at all the collocation points in that element, except for the lower boundary of the first element in each domain. At these boundary points the boundary conditions apply. The elements are constructed such that, in the unbudded cells domain the lower boundary of the first element starts at mass zero and the upper boundary of the last element coincides with the transition mass. In the budded cells domain, the lower boundary of the first element starts at age zero and the upper bound of the last element ends at some age, $a_\infty$, which is reasonably larger than the upper limit of the critical age of division. Consequently, the method approximates the partial differential equations (PBEs) by coupled sets of ordinary differential equations (ODEs) for the values of the cell mass and cell age distributions at the collocation points. Integral terms are approximated by Gaussian quadrature [11]. The resulting ODEs at the collocation points $m_i$ in the unbudded domain and $a_i$ in the budded domain are:

\[
\frac{dW_{ui}}{dt} = -\sum_{j=2}^{N_u} r(S, S_{e}) A_{u(i,j)} W_{uj} + 2\sum_{j=1}^{N_b} w_{gbj} \Gamma_j W_{bj} P_{(i,j)} - D W_{ui} \tag{4.17}
\]

\[
\frac{dW_{bi}}{dt} = -\sum_{j=1}^{N_b} A_{b(i,j)} W_{bj} - (D + \Gamma_i) W_{bi} \tag{4.18}
\]

where $W_{ui}$ is the value of the cell mass distribution at $m_i$, $W_{bi}$ is the value of the cell age distribution at $a_i$, $N_u$ and $N_b$ are the total number of collocation points in
the unbudded and budded domains, \(A_{u(i,j)}\) and \(A_{b(i,j)}\) are the first derivative weight matrices in the unbudded and budded domains, \(wg_{bi}\) is the quadrature weight in the budded domain at \(a_i\). Finlayson (1980) illustrated the detailed derivation and evaluation of the weight matrices for first and second order derivatives as well as the evaluation of the quadrature weights. \(\Gamma_i\) is the division intensity function at \(a_i\), \(P(i,j)\) is the value of the birth probability function at \(m_i\) and \(a_j\), \(P(m_i,a_j)\). The boundary conditions are now:

\[
W_{u1} = 0 \tag{4.19}
\]

\[
W_{b1} = r(S, S_e)W_{u(N_u)} \tag{4.20}
\]

The substrate balance becomes:

\[
\frac{dS}{dt} = D(S_f - S) - \sum_{i=2}^{N_u} \frac{1}{\lambda_1} r(S, S_e)wg_{ui}W_{ui} - \sum_{i=1}^{N_b} \frac{1}{\lambda_2(S, S_e)}wg_{bi}W_{bi} \tag{4.21}
\]

The rest of the model equations remain the same.

### 4.4 Model Simulations

Model simulations were performed using MATLAB. The model equations were solved using ODE15s, a stiff ODE solver. An analytical expression of the Jacobian matrix was supplied to the solver. The method of orthogonal collocation on finite elements was used to solve the model equations. Two fixed meshes of 16 elements each were used to discretize the budded and unbudded cell domains. Each
element had 3 internal collocation points obtained as the roots of the appropriate Jacobi polynomials [40]. The total number of collocation points in either domain, $N_u$ or $N_b$, was 65. The state vector of the resulting ODE model consists of the cell mass distribution at each collocation point in the unbudded domain, the cell age distribution at each collocation point in the budded domain, the substrate, the first and second order filtered substrates, as well as, an auxiliary mass variable used to calculate the increment of cell mass due to single cell growth rate. In the unbudded cells domain, the elements are positioned such that the first element extends from mass zero to $m = (m_{omin} - 0.2)$, where $m_{omin}$ is the average value of the minimum mass of a newborn daughter cell, $m_{omin} = m_t - m_s$. The rest of the elements are equally divided and they extend from the end of the first element to the transition mass. The reason for selecting this distribution of elements is that the probability of having a cell born with a mass significantly lower than $m_{omin}$ is very slim except for birth probability functions that are too broad. Therefore, one element is enough to describe this region for most probability functions with reasonably narrow bimodal distributions. However, there is a very good probability that a cell will be born with a mass anywhere between $m_{omin}$ and $m_t$, which dictates distributing the rest of the elements equally in this region. The budded cells domain is divided into two regions, namely, the pre-division and the division regions. The pre-division region extends from age zero to the minimum critical age of division and comprise of one sixth of the elements in the budded domain. The rest of the elements equally partitions the
division region, which extends from the minimum critical age of division to 0.7 hr beyond the maximum critical age of division.

In order to proceed with the solution a set of initial conditions should be specified including masses of budded cells at the age collocation points. At this point, the critical age of division is calculated and subsequently the division intensity function is determined at each collocation point. Given the masses of the budded cells the birth probability matrix, \( P(i,j) \), is evaluated and the equations can then be integrated. It should be noted, however, that the single cell growth rate, the division intensity function and the probability matrix vary with time, therefore, it is necessary to update these functions during the integration. In order to calculate the new probability matrix at \( t = \Delta t \) (assuming that we start at \( t = 0 \)), the masses of the budded cells at the age collocation points should be updated. The increment of mass of a cell at age \( a_i \) during \( \Delta t \) can be calculated by integrating the single cell growth rate, equation 4.11. However, the updated mass is no longer the cell mass at age \( a_i \), instead it is the mass of a cell at age \( a_i + \Delta t \). Hence, Lagrange interpolating polynomials were used [7] to recalculate the masses at the original age collocation points. A flowchart illustrating the solution procedure in shown in figure 4.4. The parameter values used in the numerical simulations are summarized in table 4.1. These values were used in all the simulations discussed in this chapter unless otherwise stated.
Assuming constant growth conditions for all previous times (at $S$ and $S''$), calculate the mass of budded cells at the age collocation points, $m_b(a_i)$.

- Initialize the time variable to zero, $t = 0$.
- Initialize the auxiliary mass variable to zero, $m^\tau = 0$.
- Specify the initial state vector, $x_0$, as the initial cell mass distribution of unbudded cells, cell age distribution of budded cells, initial substrate concentration, filtered substrates concentrations and $m^\tau$.

Figure 4.2: Flowchart illustration of the solution procedure for the hybrid mass-age distribution model

(part a)
(figure continued)
Define the system of algebraic equations and ordinary differential equations:

Algebraic loop:
- Operating conditions; dilution rate, D(t), and substrate feed concentration, S_i(t).
- Cell age distribution at \( a_0 = 0 \), \( W_b(0) \).
- Cell mass distribution at \( m_0 = 0 \), \( W_u(0) \).
- \( m_b(a, i, a_{od}, S') \), \( \Gamma(a, a_{od}) \), \( P(m, m_b(a, i)) \) and \( r(S,S') \)

ODEs:
- Population balances at the mass collocation points.
- Population balances at the age collocation points.
- Substrate balance.
- Filtered substrates balances.
- The auxiliary mass growth equation.

Define the elements of the Jacobian matrix

Invoke the ODE stiff solver, ODE15s, to integrate the system of equations starting at \( x_0 \) from time \( t \) to \( t + \Delta t \).

Choose the option that the analytical Jacobian matrix is available.

Update the output vectors by the values of system variables at the end of the integration (time = \( t + \Delta t \)).

Re-initialize the auxiliary mass variable to zero, \( m^- = 0 \).
Update the initial state vector, \( x_0 \), to the values of the cell mass distribution of unbudded cells, cell age distribution of budded cells, substrate concentration, filtered substrates concentrations at \( t + \Delta t \), and \( m^- \).
Update the time variable to \( t = t + \Delta t \).

\( \text{Is } t > t_{sim} \)?

No

Yes

Plot results

Finish

(part b)
Table 4.1: Parameters of the Hybrid Mass-Age Distribution Model

<table>
<thead>
<tr>
<th>General Model Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>( m_s ) 3 ng</td>
</tr>
<tr>
<td>( m_t ) 4 ng</td>
</tr>
<tr>
<td>( S_{\text{min}} ) 0.25 g/L</td>
</tr>
<tr>
<td>( \alpha_0 ) 2.5 l/hr</td>
</tr>
<tr>
<td>( \lambda_1 ) 100 ng\text{biomass cell/g substrate}</td>
</tr>
<tr>
<td>( \pi_0 ) 1.0 hr</td>
</tr>
<tr>
<td>( \pi_1 ) 0.5 hr g/L</td>
</tr>
<tr>
<td>( \pi_2 ) 3.0 hr g/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters of the Single Cell Growth Rate Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_1 ) 2.5 g/L</td>
</tr>
<tr>
<td>( K_2 ) 0.001 g/L</td>
</tr>
<tr>
<td>( \mu ) 1.65 ng/hr</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters of the Division Intensity Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n_d ) 2</td>
</tr>
<tr>
<td>( \alpha_d ) 2000 l/hr(^3)</td>
</tr>
<tr>
<td>( \Gamma_{\text{max}} ) 40 l/hr</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters of the Birth Probability Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha ) 4.0</td>
</tr>
<tr>
<td>( \beta ) 200</td>
</tr>
</tbody>
</table>

4.4.1 Analytical Jacobian Matrix

The analytical Jacobian matrix was provided to the stiff ODE solver. The number of ODEs in the unbudded and budded regions were \((N_u - 1)\) and \((N_b - 1)\), respectively, because the cell mass and cell age distributions at the first collocation point in each domain were determined using the boundary conditions. In addition to the \((N_u + N_b - 2)\) equations, the system consists of one substrate balance equation, two filtered substrate equations and an auxiliary equation used to determine the increment of mass in budded cells. Therefore, the total number of equations is \(N_t = N_u + N_b + 2\). The \((i,j)\) component of the Jacobian matrix, \(J_{(i,j)}\), is the partial

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
derivative of the $i^{th}$ ODE with respect to the $j^{th}$ dependent variable. The equations are arranged such that the first $N_u - 1$ equations are the discretized PBE in the unbudded region, followed by $N_b - 1$ equations representing the discretized PBE in the budded region, followed by the substrate balance, equation $N_t - 3$, then the first and second order filtered substrates, equations $N_t - 2$ and $N_t - 1$, and finally the auxiliary mass growth rate, equation $N_t$.

The derivation of each element in the Jacobian matrix is illustrated in the rest of this section. Therefore, the remaining parts of this section are intended only for the readers who are interested in the full derivation of the Jacobian matrix. The rest of the readers may find this chapter more readable if they skip the remainder of this section to the next one. In the following parts of this section, $f_i$ is the right hand side of the differential equation $I$. The index $I$ always refer to the number of the differential equation, and therefore, it varies from 1 to $N_t$. The discretized form of the PBE at the mass collocation points 2 to $N_u$ are given by the equations 1 to $(N_u - 1)$. For simplicity, an index $i$ was defined such that $i = I + 1$. Therefore, in equations 1 to $(N_u - 1)$, while $I$ refers to the ODE number, $i$ refers to the mass collocation point at which this equation applies. Likewise, the discretized form of the PBE at the age collocation points 2 to $N_b$ are given by equations $N_u$ to $(N_u + N_b - 2)$. Therefore, the auxiliary index $i$ in these equations was defined such that $i = I - N_u + 2$. Again, while $I$ refers to the ODE number, $i$ refers to the age collocation point at which the equation applies.
Unbudded Cells Domain, equations ($l = 1$ to $(N_u - 1)$)

The ODEs at the mass collocation points in the unbudded cells domain are given by:

\[
 f_l = -r \sum_{j=2}^{N_u} A_{u(i,j)} W_{uj} + 2 \sum_{j=1}^{N_b} w_{bj} \Gamma_j W_{bj} P_{(i,j)} - D W_{ui} 
\]

\[
 \Rightarrow f_l = -r \sum_{j=2}^{N_u} A_{u(i,j)} W_{uj} + 2 \sum_{j=2}^{N_b} w_{bj} \Gamma_j W_{bj} P_{(i,j)} - D W_{ui} 
\]

\[
 + 2 w_{b1} \Gamma_1 W_{b1} P_{(i,1)} 
\]

\[
 \Rightarrow f_l = -r \sum_{j=2}^{N_u} A_{u(i,j)} W_{uj} + 2 \sum_{j=2}^{N_b} w_{bj} \Gamma_j W_{bj} P_{(i,j)} - D W_{ui} 
\]

\[
 + 2 r w_{b1} \Gamma_1 W_{u(N_u)} P_{(i,1)} 
\]

However, $\Gamma_1$ is the division intensity at age zero which is identically 0, therefore,

\[
 f_l = -r \sum_{j=2}^{N_u} A_{u(i,j)} W_{uj} + 2 \sum_{j=2}^{N_b} w_{bj} \Gamma_j W_{bj} P_{(i,j)} - D W_{ui} 
\]

(4.23)

The partial derivatives with respect to the cell mass distributions in the unbudded cells domain are:

For $k = 2$ to $N_u$

\[
 J_{(l,k-1)|_{(k \neq i)}} = \frac{\partial f_l}{\partial W_{uk}}|_{(k \neq i)} = -r A_{u(i,k)} 
\]

(4.24)

\[
 J_{(l,k-1)|_{(k=i)}} = \frac{\partial f_l}{\partial W_{uk}}|_{(k=i)} = -r A_{u(k,k)} - D 
\]

(4.25)
The partial derivatives with respect to the cell age distributions in the budded cells domain are:

For \( k = 2 \) to \( N_b \)

\[
J_{(l,k+N_u-2)} = \frac{\partial f_l}{\partial W_{bk}} = 2w g_{bk} \Gamma_k P_{(i,k)} \tag{4.26}
\]

The partial derivatives with respect to the substrate concentration, \( S \):

\[
J_{(l,N_t-3)} = \frac{\partial f_l}{\partial S} = -\frac{dr}{dS} \sum_{j=2}^{N_u} A_{u(i,j)} W_{uj} \tag{4.27}
\]

The partial derivatives with respect to the first order filtered substrate concentration, \( S^{(1)} \):

\[
J_{(l,N_t-2)} = \frac{\partial f_l}{\partial S^{(1)}} = 0 \tag{4.28}
\]

The partial derivatives with respect to the effective substrate concentration, \( S_e \):

\[
J_{(l,N_t-1)} = \frac{\partial f_l}{\partial S_e} = -\frac{dr}{dS_e} \sum_{j=2}^{N_u} A_{u(i,j)} W_{uj} + 2 \sum_{j=2}^{N_u} w g_{bj} \frac{d\Gamma_j}{dS_e} W_{bj} P_{(i,j)} \tag{4.29}
\]

The partial derivatives with respect to auxiliary mass, \( \tilde{m} \):

\[
J_{(l,N_t)} = \frac{\partial f_l}{\partial \tilde{m}} = 0 \tag{4.30}
\]
where the functions \( \frac{dr}{dS_e} \), \( \frac{dr}{dS_e} \) and \( \frac{d\Gamma_i}{dS_e} \) are given by

\[
\frac{dr}{dS} = \frac{\mu S_e}{(K_1 + S_e)(K_2 + S)^2}
\]

(4.31)

\[
\frac{dr}{dS_e} = \frac{\mu K_1 S}{(K_1 + S_e)^2 (K_2 + S)}
\]

(4.32)

\[
\frac{d\Gamma_i}{dS_e} = \frac{d\Gamma_i}{da_{cd}} \frac{da_{cd}}{dS_e}
\]

(4.33)

where

\[
\frac{d\Gamma_i}{da_{cd}} = \frac{d\Gamma(a_i, S_e)}{da_{cd}} = \begin{cases} 
0 & a_i \leq a_{cd}(S_e) \\
-n_d(a_{cd}(a - a_{cd}(S_e))^n a - 1) & a_{cd}(S_e) < a_i \leq a_{cd}(S_e) + \delta_d \\
0 & a_i > a_{cd}(S_e) + \delta_d 
\end{cases}
\]

(4.34)

and

\[
\frac{da_{cd}}{dS_e} = \begin{cases} 
-\frac{n_d}{2} & S_e > S_{min} \\
0 & S_e \leq S_{min}
\end{cases}
\]

(4.35)

**Budded Cells Domain, equations (l = N_u to (N_u + N_b - 2))**

The ODEs at the age collocation points in the budded cells domain are given by:

\[
f_l = -\sum_{j=1}^{N_b} A_{b(i,j)} W_{bj} - (D + \Gamma_i) W_{bi}
\]

\[
\Rightarrow f_l = -\sum_{j=2}^{N_b} A_{b(i,j)} W_{bj} - A_{b(i,1)} W_{b1} - (D + \Gamma_i) W_{bi}
\]

(4.36)

\[
\Rightarrow f_l = -\sum_{j=2}^{N_b} A_{b(i,j)} W_{bj} - r A_{b(i,1)} W_{u(N_u)} - (D + \Gamma_i) W_{bi}
\]
The partial derivatives with respect to the cell mass distributions in the unbudded cells domain are:

For $k = 2$ to $(N_u - 1)$

$$J_{(l,k-1)} = \frac{\partial f_i}{\partial W_{uk}} = 0 \quad (4.37)$$

At $k = N_u$

$$J_{(l,k-1)} = \frac{\partial f_i}{\partial W_{u(N_u)}} = -\tau A_b(i,1) \quad (4.38)$$

The partial derivatives with respect to the cell age distributions in the budded cells domain are:

For $k = 2$ to $N_b$

$$J_{(l,k+N_u-2)}|_{k\neq i} = \frac{\partial f_i}{\partial W_{bk}}|_{k\neq i} = -A_b(i,k) \quad (4.39)$$

$$J_{(l,k+N_u-2)}|_{k=i} = \frac{\partial f_i}{\partial W_{bk}}|_{k=i} = -A_b(k,k) - D - \Gamma_k \quad (4.40)$$

The partial derivatives with respect to the substrate concentration, $S$:

$$J_{(l,Nt-3)} = \frac{\partial f_i}{\partial S} = -\frac{d\tau}{dS}A_b(i,1)W_u(N_u) \quad (4.41)$$

The partial derivatives with respect to the first order filtered substrate concentration, $S^{(1)}$:

$$J_{(l,Nt-2)} = \frac{\partial f_i}{\partial S^{(1)}} = 0 \quad (4.42)$$
The partial derivatives with respect to the effective substrate concentration, $S_e$:

$$J_{(l,N_t-1)} = \frac{\partial f_l}{\partial S_e} = -\frac{d\tau}{dS_e} A_{b(l,1)} W_{u(N_u)} - \frac{d\Gamma_l}{dS_e} W_{bi} \quad (4.43)$$

The partial derivatives with respect to auxiliary mass, $\tilde{m}$:

$$J_{(l,N_t)} = \frac{\partial f_l}{\partial \tilde{m}} = 0 \quad (4.44)$$

**Substrate Balance, equation $(N_t - 3)$**

The substrate balance is:

$$f_{(N_t-3)} = D(S_f - S) - \sum_{i=2}^{N_u} \frac{1}{\lambda_1} r(S, S_e) w_{gi} W_{ui} - \sum_{i=1}^{N_b} \frac{1}{\lambda_2(S, S_e)} w_{bi} W_{bi}$$

$$\Rightarrow f_{(N_t-3)} = D(S_f - S) - \frac{r}{\lambda_1} \sum_{i=2}^{N_u} w_{gi} W_{ui} - \frac{r}{\lambda_1} \sum_{i=2}^{N_b} w_{bi} W_{bi} - \frac{r}{\lambda_1} (w_{b1} W_{b1})$$

$$\Rightarrow f_{(N_t-3)} = D(S_f - S) - \frac{r}{\lambda_1} \sum_{i=2}^{N_u} w_{gi} W_{ui} - \frac{r}{\lambda_1} \sum_{i=2}^{N_b} w_{bi} W_{bi} - \frac{r^2}{\lambda_1} (w_{b1} W_{u(N_u)}) \quad (4.45)$$

The partial derivatives with respect to the cell mass distributions in the unbudded cells domain are:

For $k = 2$ to $(N_u - 1)$

$$J_{(N_t-3,k-1)} = \frac{\partial f_{(N_t-3)}}{\partial W_{uk}} = -\frac{r}{\lambda_1} w_{uk} \quad (4.46)$$
At $k = N_u$

\[
J_{(N_t-3,k-1)} = \frac{\partial f(N_t-3)}{\partial W_{u(N_u)}} = -\frac{r}{\lambda_1} w_{gu(N_u)} - \frac{r^2}{\lambda_1} w_{gb1} \tag{4.47}
\]

The partial derivatives with respect to the cell age distributions in the budded cells domain are:

For $k = 2$ to $N_b$

\[
J_{(N_t-3,k+N_u-2)} = \frac{\partial f(N_t-3)}{\partial W_{bk}} = -\frac{r}{\lambda_1} w_{gbk} \tag{4.48}
\]

The partial derivative with respect to the substrate concentration, $S$:

\[
J_{(N_t-3,N_t-3)} = \frac{\partial f(N_t-3)}{\partial S} = D - \frac{1}{\lambda_1} \frac{dr}{ds} \left( \sum_{i=2}^{N_u} w_{gu_i} W_{ui} + \sum_{i=2}^{N_b} w_{gbi} W_{bi} + 2r(w_{gb1} W_{u(N_u)}) \right) \tag{4.49}
\]

The partial derivative with respect to the first order filtered substrate concentration, $S^{(1)}$:

\[
J_{(N_t-3,N_t-2)} = \frac{\partial f(N_t-3)}{\partial S^{(1)}} = 0 \tag{4.50}
\]

The partial derivative with respect to the effective substrate concentration, $S_e$:

\[
J_{(N_t-3,N_t-1)} = \frac{\partial f(N_t-3)}{\partial S_e} = -\frac{1}{\lambda_1} \frac{dr}{ds} \left( \sum_{i=2}^{N_u} w_{gu_i} W_{ui} + \sum_{i=2}^{N_b} w_{gbi} W_{bi} + 2r(w_{gb1} W_{u(N_u)}) \right) \tag{4.51}
\]
The partial derivative with respect to auxiliary mass, \( \bar{m} \):

\[
J_{(N_t-3,N_t)} = \frac{\partial f_{(N_t-3)}}{\partial \bar{m}} = 0 \quad (4.52)
\]

**Filtered Substrates, equations** \((l = (N_t - 2) \text{ to } (N_t - 1))\)

The filtered substrate equations are:

\[
f_{(N_t-2)} = \alpha_a(S - S^{(1)}) \quad (4.53)
\]

\[
f_{(N_t-1)} = \alpha_a(S^{(1)} - S_e) \quad (4.54)
\]

The partial derivatives with respect to the cell mass distributions in the unbudded cells domain are:

For \( k = 2 \text{ to } N_u \)

\[
J_{(l,k-1)} = \frac{\partial f_l}{\partial W_{uk}} = 0 \quad (4.55)
\]

The partial derivatives with respect to the cell age distributions in the budded cells domain are:

For \( k = 2 \text{ to } N_b \)

\[
J_{(l,k+N_u-2)} = \frac{\partial f_l}{\partial W_{bk}} = 0 \quad (4.56)
\]
The partial derivatives with respect to the substrate concentration, $S$:

$$J_{(N_t-2,N_t-3)} = \frac{\partial f_{(N_t-2)}}{\partial S} = \alpha_a$$  \hspace{1cm} (4.57)

$$J_{(N_t-1,N_t-3)} = \frac{\partial f_{(N_t-1)}}{\partial S} = 0$$  \hspace{1cm} (4.58)

The partial derivatives with respect to the first order filtered substrate concentration, $S^{(1)}$:

$$J_{(N_t-2,N_t-2)} = \frac{\partial f_{(N_t-2)}}{\partial S^{(1)}} = -\alpha_a$$  \hspace{1cm} (4.59)

$$J_{(N_t-1,N_t-2)} = \frac{\partial f_{(N_t-1)}}{\partial S^{(1)}} = \alpha_a$$  \hspace{1cm} (4.60)

The partial derivatives with respect to the effective substrate concentration, $S_e$:

$$J_{(N_t-2,N_t-1)} = \frac{\partial f_{(N_t-2)}}{\partial S_e} = 0$$  \hspace{1cm} (4.61)

$$J_{(N_t-1,N_t-1)} = \frac{\partial f_{(N_t-1)}}{\partial S_e} = -\alpha_a$$  \hspace{1cm} (4.62)

The partial derivatives with respect to auxiliary mass, $\tilde{m}$:

$$J_{(t,N_t)} = \frac{\partial f_t}{\partial \tilde{m}} = 0$$  \hspace{1cm} (4.63)
Auxiliary Mass Equation, equation $N_t$

The auxiliary mass equation is:

$$f_{(N_t)} = \frac{d\tilde{m}}{dt} = r(S, S_e) = \frac{\mu S_e}{(K_1 + S_e)(K_2 + S)}$$  \hspace{1cm} (4.64)

The partial derivatives with respect to the cell mass distributions in the unbudded cells domain are:

For $k = 2$ to $N_u$

$$J_{(N_t,k-1)} = \frac{\partial f_{(N_t)}}{\partial \tilde{W}_{uk}} = 0$$  \hspace{1cm} (4.65)

The partial derivatives with respect to the cell age distributions in the budded cells domain are:

For $k = 2$ to $N_b$

$$J_{(N_t,k+N_u-2)} = \frac{\partial f_{(N_t)}}{\partial \tilde{W}_{bk}} = 0$$  \hspace{1cm} (4.66)

The partial derivative with respect to the substrate concentration, $S$:

$$J_{(N_t,N_t-3)} = \frac{\partial f_{(N_t)}}{\partial S} = \frac{dr}{dS}$$  \hspace{1cm} (4.67)

The partial derivative with respect to the first order filtered substrate concentration, $S^{(1)}$:

$$J_{(N_t,N_t-2)} = \frac{\partial f_{(N_t)}}{\partial S^{(1)}} = 0$$  \hspace{1cm} (4.68)
The partial derivative with respect to the effective substrate concentration, $S_e$:

$$J_{(N_t-1)N_t} = \frac{\partial f_{(N_t)}}{\partial S_e} = \frac{dr}{dS_e}$$  \hspace{1cm} (4.69)

The partial derivative with respect to auxiliary mass, $m$:

$$J_{(N_t,N_t)} = \frac{\partial f_{(N_t)}}{\partial m} = 0$$  \hspace{1cm} (4.70)

### 4.4.2 Steady State Analysis

Determining the steady state distribution analytically can be used as a tool to verify model simulations. The procedure is straightforward: first the analytical steady state distribution is determined; and then the eigenvalues of the Jacobian matrix are found at the steady state. If all the real parts of the Jacobian matrix eigenvalues are negative then the steady state is stable. In this case, starting the simulations at or relatively near the steady state distribution, the solution should eventually approach the original steady state distribution. However, if one or more of the eigenvalues real parts are positive then the steady state is unstable. Therefore, starting the simulation with initial conditions that slightly deviate from the steady state should be sufficient for the solution to eventually deviate from this steady state and approach another state. The final state might be another steady state, an oscillatory state or possibly a chaotic attractor.
At steady state all the derivatives and partial derivatives with respect to time are identically zero except for the auxiliary mass equation which can be managed as the derivative of cell mass with respect to cell age. This equation is actually decoupled from the other system equations. In the current model, the rate of mass increase is independent of cell mass and thus at steady state the single cell growth rate is constant, but it is not zero as will be shown below.

The steady state PBEs are

$$\frac{d(rW_u(m))}{dm} = -DW_u(m) + 2 \int_0^\infty P(m, a) \Gamma(a) W_b(a) da$$ \hspace{1cm} (4.71)

$$\frac{dW_b(a)}{da} = -(D + \Gamma(a))W_b(a)$$ \hspace{1cm} (4.72)

The boundary conditions associated with these equations at steady state are:

$$W_u(0) = 0$$ \hspace{1cm} (4.73)

$$W_b(0) = rW_u(m_t)$$ \hspace{1cm} (4.74)

At steady state \(\frac{ds}{dt} = \frac{ds(1)}{dt} = \frac{ds_e}{dt} = 0\), therefore, the substrate concentration is constant and both the effective and the first order filtered substrate are equal to the steady state substrate concentration. This fixes the critical age of division, and consequently the division intensity function becomes a function of age only. Also the single cell growth rate becomes fixed. If \(S_{st}\) denotes the steady state substrate
concentration then \( a_{cd} \), \( \Gamma(a) \) and \( r \) become:

\[
a_{cd} = \begin{cases} 
\pi_0 + \frac{\pi_1}{S_{st}} & S_{st} > S_{\text{min}} \\
\pi_2 & S_{st} \leq S_{\text{min}}
\end{cases} \quad (4.75)
\]

\[
\Gamma(a) = \begin{cases} 
0 & a \leq a_{cd} \\
\alpha_d(a - a_{cd})^{n_d} & a_{cd} < a \leq a_{cd} + \delta_d \\
\Gamma_{\text{max}} & a > a_{cd} + \delta_d
\end{cases} \quad (4.76)
\]

\[
r = \frac{\mu S_{st}^2}{(K_1 + S_{st})(K_2 + S_{st})} \quad (4.77)
\]

Since \( r \) is now constant a direct relation can be found between the budded cell mass and its age. This is possible because in this simplified model all cells start budding exactly at the same mass, \( m_t \). Using equation 4.11

\[
m_b(a) = m_t + ra \quad (4.78)
\]

Hence, the probability function now becomes an explicit function of \( m \) and \( a \):

\[
P(m, a) = \alpha(\exp(-\beta(m_s - m)^2) + \exp(-\beta((m_t + ra - m_s) - m)^2)) \quad (4.79)
\]

The solution for the steady state distribution is obtained by trial and error. It proceeds as follows. At specific operating conditions (dilution rate and substrate feed concentration) and for a specific set of system parameters, a value of the steady state
substrate concentration is assumed. The critical age of division is then calculated from equation 4.75, as well as the single cell growth rate from equation 4.77. The budded cells age distribution can be found by integrating equation 4.72 using an integrating factor:

\[
W_b(a) = W_b(0) \exp(- \int_0^a \left(D + \Gamma(a)\right) da)
\]

(4.80)

Using equation 4.76 the integral of \(\Gamma(a)\) with respect to \(a\) is given by

\[
\int_0^a \Gamma(a) = \\
\begin{cases} 
0 & a \leq a_{cd} \\
\frac{\alpha_d}{n_d+1}(a - a_{cd})^{(n_d+1)} & a_{cd} < a \leq a_{cd} + \delta_d \\
\frac{\alpha_d}{n_d+1}\delta_d^{(n_d+1)} + \Gamma_{max}(a - (a_{cd} + \delta_d)) & a > a_{cd} + \delta_d
\end{cases}
\]

(4.81)

For the purpose of simplicity, the following constants are defined at this point:

\(c_1 = \frac{\alpha_d}{n_d+1}, c_2 = a_{cd} + \delta_d\) and \(c_3 = m_t - m_s\). The budded cells age distribution become:

\[
W_b(a) = W_b(0)e^{-Da} \begin{cases} 
1 & a \leq a_{cd} \\
\exp(-c_1(a - a_{cd})^{(n_d+1)}) & a_{cd} < a \leq c_2 \\
\exp(\Gamma_{max}c_2 - c_1\delta_d^{(n_d+1)}) \exp(-\Gamma_{max}a) & a > c_2
\end{cases}
\]

(4.82)

The steady state cell mass distribution for the unbudded cells can now be obtained by plugging the expression for \(W_b(a)\) above in equation 4.71 and then integrating it with respect to mass. It must be noted that the second term on the
LHS of equation 4.71 is a function of \( m \) only. To proceed, define the function 
\[ g_1(m, a) = P(m, a) \Gamma(a) W_b(a), \]
then
\[
g_1(m, a) = \begin{cases} 
\alpha W_b(0) e^{-D_a (e^{-\beta (m_0 - m)^2} + e^{-\beta (c_3 + r_0 - m)^2})} & a \leq a_{cd} \\
0 & a_{cd} < a \leq c_2 \\
\alpha_d (a - a_{cd}) e^{-c_1 (a - a_{cd}) (n_d + 1)} & a_{cd} < a \leq c_2 \\
\Gamma_{max} e^{(\Gamma_{max} c_2 - c_1 \delta_d (n_d + 1)) e^{-\Gamma_{max}} a} & a > c_2
\end{cases}
\] (4.83)

To simplify the expressions define the following constants: \( c_5 = \alpha \alpha_d \) and 
\( c_6 = \alpha \Gamma_{max} e^{(\Gamma_{max} c_2 - c_1 \delta_d (n_d + 1))} \), also define the functions \( h_1(a) \), \( h_2(m, a) \), \( h_3(a) \) and 
\( h_4(m, a) \) such that:

\[
h_1(a) = (a - a_{cd}) e^{-D_a e^{-c_1 (a - a_{cd}) (n_d + 1)}}
\] (4.84)

\[
h_2(m, a) = h_1(a) e^{-\beta (c_3 + r_0 - m)^2}
\] (4.85)

\[
h_3(a) = e^{-(D + \Gamma_{max}) a}
\] (4.86)

\[
h_4(m, a) = h_3(a) e^{-\beta (c_3 + r_0 - m)^2}
\] (4.87)
It can be verified that \( g_1(m, a) \) is simply

\[
g_1(m, a) = W_b(0) \begin{cases} 
0 & a \leq a_{cd} \\
c_5[e^{-\beta(m-m)^2}h_1(a) + h_2(m, a)] & a_{cd} < a \leq c_2 \\
c_6[e^{-\beta(m-m)^2}h_3(a) + h_4(m, a)] & a > c_2 
\end{cases}
\]  \hspace{1cm} (4.88)

Our objective is to evaluate the second term on the LHS of equation 4.71. Consider the function \( G_1(m) = \frac{\int_0^\infty g_1(m, a)da}{W_b(0)} \), this function can be written as:

\[
G_1(m) = \begin{align*}
c_5(e^{-\beta(m-m)^2} & \int_{a_{cd}}^{c_2} h_1(a)da + \int_{a_{cd}}^{c_2} h_2(m, a)da \\
+ c_6(e^{-\beta(m-m)^2} & \int_{c_2}^{\infty} h_3(a)da + \int_{c_2}^{\infty} h_4(m, a)da)
\end{align*}
\]  \hspace{1cm} (4.89)

The first two integrals are complicated and there is probably no closed form solution for such expressions. However, satisfactory numerical values can be obtained by numerical integration of these two terms. On the other hand, analytical closed form solutions of the latter couple of integrals are possible and they are given below without further discussion.

\[
\int_{c_2}^{\infty} h_3(a)da = \frac{\exp(-D + \Gamma_{max})c_2}{(D + \Gamma_{max})}
\]  \hspace{1cm} (4.90)

\[
\int_{c_2}^{\infty} h_4(m, a)da = 0.5\sqrt{\pi}e^{-\beta(c_3-m)^2-0.25\frac{d_1(m)^2}{d_2}} \left( 1 - \text{erf} \left( \frac{c_2\sqrt{-d_2} - 0.5\frac{d_1(m)}{\sqrt{-d_2}}}{\sqrt{-d_2}} \right) \right)
\]  \hspace{1cm} (4.91)
where $d_1(m) = -[D + \Gamma_{\text{max}} + 2\beta(c_3 - m)r]$ and $d_2 = -\beta r^2$. Equation 4.71 can now be written as:

$$\frac{d(W_u(m))}{dm} + \frac{D}{r} W_u(m) = \frac{2}{r} W_b(0) G_1(m)$$

(4.92)

The solution of equation 4.92 can be obtained using an integrating factor. Considering the boundary condition equation 4.73, the solution obtained is

$$W_u(m) = \frac{2}{r} W_b(0) e^{-\frac{2}{r} m} \int_0^m G_1(x) dx$$

(4.93)

At this point, the budded cells steady state age distribution and unbudded cells steady state mass distribution are known up to a constant multiplier, $W_b(0)$, equations 4.82 and 4.93. In order to determine the value of $W_b(0)$ the steady state substrate balance should be used. However, the substrate concentration used in the calculations so far is just a guess. Hence, it is reasonable to try and check the validity of this guess as early as possible in the solution in order to avoid unnecessary repetitions of tedious calculations, which is time consuming. Fortunately, the validity of the substrate concentration guess can be determined at this point of the solution without even knowing the actual value of $W_b(0)$. The validation of the initial guess of $S_{st}$ proceeds as follows. Plugging equation 4.93 in the second
boundary condition, equation 4.74, the following equality condition is obtained.

\[ 2W_b(0)e^{-\frac{\rho}{m_1}} \int_0^{m_1} G_1(x) dx = W_b(0) \]  
\[ \Rightarrow 2e^{-\frac{\rho}{m_1}} \int_0^{m_1} G_1(x) dx = 1 \]  

(4.94)

The equation above provides simple means for determining whether or not the assumed steady state substrate concentration is a good guess. The LHS of equation 4.94 can be numerically evaluated. The value of the LHS is then compared to unity. If the LHS is greater than unity, then the actual steady state substrate concentration is less than the assumed concentration and vice versa. Once the actual steady state substrate concentration is determined, the value of \( W_b(0) \) can be found. The steady state substrate balance yields:

\[ D(S_f - S_{st}) = \frac{\tau}{\lambda_1} \left[ \int_0^{m_1} W_u(m) dm + \int_0^\infty W_b(a) da \right] \]  

(4.95)

Substituting equations 4.82 and 4.93 into equation 4.95 gives:

\[ D(S_f - S_{st}) = \frac{\tau}{\lambda_1} W_b(0) \left[ \int_0^{m_1} e^{-\frac{\rho}{m_1}} \int_0^{m_1} G_1(x) dx dm \right. \]
\[ + \int_0^{a_{cd}} e^{-Da} da + \int_0^{a_{cd}} e^{-Da} \exp(-c_1(a - a_{cd})^{(n_{cd}+1)}) da \]
\[ + \exp(\Gamma_{max} c_2 - c_1 \delta_d^{(n_{cd}+1)}) \int_0^\infty e^{-(D+\Gamma_{max})a} da \]  

(4.96)
All the integrals on the RHS of equation 4.96 can be evaluated either analytically or numerically. Therefore, the only unknown in equation 4.96 is $W_b(0)$, which concludes the steady state analysis of the hybrid mass-age model.

4.5 Results and Discussion

To gain confidence in the various model simulations, one might desire to examine the numerical solution procedure first. Numerical techniques are usually scrutinized by comparing their predictions to the analytical solution of some special case that is amenable to analytical methods. In the previous section the analytical solution procedure to determine the values of system variables at steady state was illustrated. Since the solution contains integrals with no known solutions a numerical evaluation of the closed form analytical solution is required. Nonetheless, the steady state solutions obtained from the hybrid model simulations can be compared to the numerical evaluation of the analytical solution for the steady state equations. Therefore, both Mathcad and Maple programs were written to determine the steady state solution at dilution rate of 0.15 hr$^{-1}$ and feed substrate concentration of 30 g/L. Conceptually, the two codes were expected to provide reasonable and comparable answers. However, for the set of parameters shown in table 4.1 both codes failed to give satisfactory answers within reasonable execution times. Nevertheless, numerical evaluations of the analytical steady state were possible at reasonable time frame by changing some of the parameter values shown in table 4.1. The altered parameters were $n_d = 3$, $\alpha_d = 800$, $\Gamma_{max} = 20$, $\alpha = 2.821$, $\beta = 100$ and $\mu = 1.3$. For the rest
of the simulations presented in this chapter the parameters shown in table 4.1 were used. The solutions from Mathcad and Maple programs revealed the steady state mass distribution of unbudded cells, age and mass distributions of budded cells, cell number concentration in each domain, and substrate concentration. The Jacobian matrix was analyzed at the steady state point and it was found that all the eigenvalues were in the left half plane indicating a stable steady state. The numerical steady state solution was then used as the initial condition for the commencement of a numerical simulation of the transient model equations. Model simulations showed that the steady state is indeed stable and that the system remains at its steady state. Negligible deviation was observed between the analytical solution and the predictions of the numerical solution. While the former estimated the values for the substrate and cell number concentrations to be 1.61 g/L and 841.37 cell/L, respectively, the numerical values obtained were 1.60 g/L and 845.03 cell/L, giving rise to relative errors of less than 1%. The steady state mass distribution of unbudded cells and age distribution of budded cells from the analytical and numerical solutions are shown in figure 4.3. This analysis indicates the sufficiency of the number of collocation points used and the efficiency of their placement strategy.

The autonomous oscillations in *S. cerevisiae* have frequently been described as spontaneous. This conception was based on the observation that if a late batch culture of *S. cerevisiae* was switched to continuous conditions in a permissive region of the oscillations, the oscillations would appear immediately. This behavior
Figure 4.3: Comparison between the numerical evaluation of the analytical steady state distributions (●) obtained using a Mathcad program and the steady state distributions predicted by the hybrid mass-age distribution model simulation obtained using MATLAB code (—). The steady state is shown in terms of the cell mass distribution of unbudded cells (a) and the cell age distribution of budded cells (b) is depicted in part (a) of figure 4.4 in terms of the evolved carbon dioxide signal. Numerical simulations of the hybrid mass-age distribution model indicated that at dilution rate of 0.095 hr\(^{-1}\) and feed substrate concentration of 30 g/L, sustained oscillations of the yeast culture are possible. In order to investigate the spontaneity in the occurrence of these oscillations, a batch culture was simulated, starting with an initial substrate concentration of 30 g/L and a relatively small cell number concentration, 50×10\(^{10}\) cells/L, arbitrarily distributed over the cell states. The substrate was consumed within the first 10 hours, at virtual time of 30 hr which resembles the time for an actual late batch culture the dilution rate was increased to 0.095 hr\(^{-1}\).
in one step with feed substrate concentration of 30 g/L. The oscillations spontaneously occurred as shown in figure 4.4 in terms of the substrate concentration and the cell number concentration. The simulated oscillations show that, despite the spontaneous appearance of the oscillations apparent transients in these oscillations persist for up to 50 hours before these oscillations assume their final amplitude and period. It was confirmed through FFT analysis that the experimentally observed oscillations are also characterized by slow transients and that these transients may take up to two days to die out [5].

The analysis of the cell size distribution of *S. cerevisiae* batch cultures showed that the cultures progressively develop a bimodal size distribution during their late batch growth. At these conditions, small daughter cells are formed due to the poor nutritional conditions, in addition, due to starvation cells stop growing and the population cell mass distribution becomes fixed. It is suggested that this highly synchronized state is, at least partially, responsible for the spontaneous appearance of the oscillations. The hybrid mass-age model accounts for the effects of the environment on the mass of newborn daughter cells and hence the formation of the observed bimodal distribution of cell mass at the stationary phase of the batch growth. Figure 4.5 shows the experimentally observed cell mass distribution and the corresponding cell mass distribution predicted by the hybrid mass-age model at the end of the batch growth dynamics shown in figure 4.4. The experimental cell mass distribution is measured electronically, and the measurement is subject to broaden-
Figure 4.4: Comparison between the experimentally observed batch growth dynamics followed by a switch to the continuous operation at the stationary phase of the batch in terms of evolved CO$_2$ (a) and the model predictions of such dynamics in terms of the substrate concentration (b) and the cell number concentration (c).

In chapter 2, the existence of two different oscillatory states and a non-oscillatory state at the same operating conditions (see figure 2.4) were confirmed through ramp experiments. Numerical simulations of the model equations also show the existence of multiple oscillatory attractors, but at different ranges of dilution rate. Each oscil-
latory attractor can be characterized by its number of subpopulations of unbudded and budded cells. A subpopulation is observed as a local maximum in the cell mass or cell age distribution. In this chapter, an attractor with N subpopulations of budded cells and M subpopulations of unbudded cells will be referred to as a N:M attractor. Model simulations indicate that the 1:3 attractor prevailed for a dilution rate range of 0.085 to 1.0 hr$^{-1}$, while the 1:5 attractor was found at dilution rates between 0.06 and 0.07 hr$^{-1}$. The sustainability of a third attractor that appeared at dilution rates near 0.05 hr$^{-1}$ is still under investigation. The preliminary results show that the third attractor, if the oscillations are indeed sustainable, is a 1:7 attractor. The oscillatory dynamics in terms of the periodic variations of the substrate
and the cell number concentration for the 1:5 and 1:3 attractors are shown in figure 4.6. The figure also show a snap shot of the subpopulation structures responsible for the observed oscillations in terms of the cell mass distributions of unbudded cells and cell age distribution of budded cells.

Throughout our previous discussions and analysis, the multiple oscillatory states arrived at in the ramp experiments were assumed to occur at the same operating conditions. In reality, one set of oscillations was obtained at $D = 0.15 \text{ hr}^{-1}$ while the other was found at $D = 0.16 \text{ hr}^{-1}$. The assumption that these oscillations occur at the same operating conditions is reasonable given that the estimated error in the measured dilution rate is 0.005 hr$^{-1}$. However, the simulation results obtained using the hybrid model predict that different oscillatory attractors may be separated by a small interval of dilution rates. This remark raises reasonable doubt about the assumption that multiple oscillatory states observed experimentally were attained at the same operating conditions indeed rather than at two different but yet close operating points that support two different attractors. Therefore, further experimental investigation with more precise measurement of the dilution rate is needed to confirm or reject the hypothesis that the observed oscillations do occur at the same operating point.

Once again, in accordance with the ramp experiments, an extended non-oscillatory state was observed at the same operating conditions that support the oscillatory dynamics. The non-oscillatory dynamics were obtained by starting at a stable steady
Figure 4.6: Typical oscillation patterns associated with the 1:5 attractor (a) and 1:3 attractor (b). At $S_f = 30 \text{ g/L}$ and $D = 0.065 \text{ hr}^{-1}$ a periodic solution that belongs to the 1:5 attractor is obtained. Oscillations are observed in all system parameters such as substrate concentration (a-1) and cell number concentration (a-2). The 1:5 attractor is characterized by one subpopulation of budded cells (a-3) and five subpopulations of unbudded cells (a-4). At $D = 0.095 \text{ hr}^{-1}$ a periodic solution that belongs to the 1:3 attractor is obtained. Oscillations are also observed in all system parameters such as substrate concentration (b-1) and cell number concentration (b-2). The 1:3 attractor is characterized by one subpopulation of budded cells (b-3) and three subpopulations of unbudded cells (b-4).
state for some continuous operating conditions, then slowly changing the dilution rate to the value at which oscillations are known to occur. A similar situation was simulated using the hybrid mass-age distribution model. Steady state was first established at dilution rate of 0.105 hr\(^{-1}\), the dilution rate was then decreased at a constant rate to 0.095 hr\(^{-1}\) in 200 hours. The system remained at the conditions achieved at the end of the ramp for almost 200 hours before the emergence of noticeable oscillatory dynamics. Indeed, tracking a slow ramp the system was kept at a quasi-steady state. However, this steady state is apparently unstable at \(D = 0.095\) hr\(^{-1}\) and the oscillations were, therefore, bound to occur eventually. Thus, in accordance with experimental evidence, the hybrid mass-age distribution model predicts that the spontaneous appearance of the oscillations or the perseverance of an extended non-oscillatory state may happen at the same set of operating conditions depending on the path through which these conditions were established. Figure 4.7 shows two different transient dynamics observed at the same dilution rate but for different initial conditions. Once again, it is recommended to perform extended ramp experiments to investigate the stability of the non-oscillatory state observed in the ramp experiments.

4.6 Conclusions

A population balance equation model of budding yeast cultures was developed in terms of cell mass distribution of unbudded cells and cell age distribution of budded cells. The model equations were numerically solved using the method of orthog-
Figure 4.7: The existence of a stable oscillatory state and an extended unstable steady state at the same operating conditions. At $D = 0.095 \text{ hr}^{-1}$, the model predicts autonomous sustained oscillations if the initial culture was a stationary phase batch culture (a), however, if the dilution rate was changed slowly from a value that does not support oscillations, at which point the culture is at steady state, to $D = 0.095 \text{ hr}^{-1}$ an extended unstable steady state is predicted (b)
of dilution rate. However, the relative closeness of the predicted ranges of dilution rates which support different attractors raised the question whether the multiple oscillatory states observed experimentally indeed occurred at the same operating conditions or at operating conditions that are close but yet different and support different oscillatory attractors. Finally, the model predicts the existence of an extended but unstable steady state at the same operating conditions that support the oscillations.
Chapter 5

Summary

Continuous cultures of *Saccharomyces cerevisiae* can exhibit autonomous and sustained oscillations under glucose limited aerobic conditions for a range of operating conditions. At some operating conditions, continuous cultures of *S. cerevisiae* were found to possess at least two oscillatory states and one steady state. This observation cannot be explained by any currently proposed distributed models and points to a segregated mechanism of these oscillations.

A small perturbation in the distribution of states of the cells in a reactor can cause the reactor to reach a steady state when an oscillatory state is otherwise attained. This suggests that the boundaries of the basins of attraction of different stable states are highly convoluted, at least at some operating conditions, resulting in these cultures becoming sensitive to small disturbances.

An age distribution PBE model was proposed as an explanation of the autonomous oscillations in budding yeast. The model was solved numerically by the method of orthogonal collocation on finite elements. The model predicts the existence of multiple oscillatory attractors, an observation that is experimentally validated. However, the model predicts these attractors at separate regions of dilution rate where in the experiments the different attractors are found at the same
operating conditions. The range of dilution rates supporting the attractors depend on values of model parameters, the proposed functionality of the transition and division intensity functions and the operating conditions.

The age distribution model also predict the existence of a stable oscillatory state and a prolonged steady state at the same operating conditions in good agreement with experimental observations. Nonetheless, there is a conflicting evidence about the stability of the steady state. The system dynamics are very slow and the apparent steady state can persist for extended periods of time. Therefore, for all practical purposes this extended unstable steady state cannot be distinguished from an experimentally observed extended steady state. As a matter of fact, it was observed that the transients resulting from a step change in the dilution rate take up to two days to die out as evidenced by FFT analysis. Therefore, the actual system itself has slow dynamics and there is no proof that the experimentally observed steady state is indeed stable.

A detailed bifurcation analysis can be a useful tool for model discrimination. For example, most distributed models predict that the oscillations appear spontaneously if the operating conditions were changed from a region that is not permissive of the oscillations to another that is permissive, regardless of the path or rate of change [21]. This prediction is supported by experiments where the bifurcation parameter (dilution rate or agitation speed) is changed in an abrupt manner such as a step change [37]. However, in the experiments where the dilution rate was changed slowly
from a value that does not support oscillations to a value that does, no oscillations
were achieved (see chapter 2). This bifurcation phenomenon, therefore, invalidates
a large number of distributed models and provides the tool to reject these models.

The proposed age distribution PBE model was based on a simplified yeast cell
cycle. Therefore, the validity of the model was limited to the validity of its con­
stitutive simplifying assumptions. The simplified cell cycle provides a satisfactory
representation of the actual cell cycle as long as the yeast culture is not starved.
Therefore, the age distribution model was not suited for simulations of batch yeast
cultures or continuous cultures operated at extremely low dilution rates. Conse­
quently, another PBE model, that has been based on a more detailed cell cycle, was
developed. The new model uses the cell mass distribution to describe un­budded
cells domain and the cell age distribution for the budded cells domain, hence, it was
called the hybrid mass-age model. The method of orthogonal collocation on finite
elements was implemented to solve the model equations.

The hybrid distribution model predicts a number of oscillatory regions at sep­
arate but very close intervals of dilution rates. In contrast, the experimentally ob­
served multiple oscillatory state occur at the same operating conditions. However,
due to the inherent errors in experimental measurements of the operating conditions
in general and the dilution rate in particular, the operating conditions where mul­
tiple oscillations are observed might not be exactly the same. Indeed, the hybrid
model strongly suggests that the multiple oscillatory states observed do not occur
exactly at the same conditions, but at slightly different conditions, that are different enough to move the system from one oscillatory attractor to another.

The hybrid model exhibits Hopf bifurcation dynamics, in the sense that the regions where oscillations are observed the steady state is unstable. However, like the age distribution model, the hybrid model predicts very slow system dynamics and unstable steady states last for very long time before the amplitude of the oscillations becomes appreciable. The achievement of either the steady state or an oscillatory state depends on the initial distributions of the population. At the stationary phase of a batch growth the model predicts a highly synchronized bimodal distribution of cells at their G1 state. In addition, the model predicts the spontaneous and autonomous appearance of the oscillations upon the switch of late batch cultures to the continuous mode of operation.

For future experimental analysis, it is recommended to investigate the stability of the observed steady state at the same operating conditions where oscillations were found to be stable. The multiple oscillatory attractors were found in different sets of experiments. Therefore, more work is needed reproduce the observed multiple states in the course of the same experiment and using the same culture. It is suggested to start with a batch culture. Then, at the stationary phase of the batch culture the reactor is switched in a step to conditions that support multiple attractors. The oscillations are supposed to appear spontaneously. After recording a sufficient number of cycles, the dilution rate is to be reduced to a value that does not support
oscillations in one step. The new conditions are to be sustained for at least two days in order for the transient dynamics to die out and for the culture to attain steady state distribution. The dilution rate is then to be increased at a certain intermediate rate to its previous value. Depending on the rate at which the dilution rate is increased a steady state or one of the two oscillatory states observed before or maybe even a new state could be observed. Repeating this experiment a number of times can yield a relation between the rate at which the dilution rate is increased and the final conditions.

Other possible experimental investigations include studying different bifurcation parameters such as the dissolved oxygen level and the substrate feed concentration. In the case of investigating the effect of the dissolved oxygen, the variation in the dissolved oxygen level can be achieved by changing either the agitational speed or the inlet air flow rate. However, utilizing either approach would modulate the mixing properties of the chemostat and consequently it would become difficult to decisively determine whether the resultant behavior is due to changing the oxygen level or altering the chemostat mixing properties. In order to overcome this problem, instead of changing the inlet gaseous flow rate, one can change its composition. This can be achieved by mixing the inlet air with an inert gas such as nitrogen such that the gaseous flow rate is constant. In a sterile fermenter, a direct relation between the dissolved oxygen level and the partial pressure of oxygen in the inlet gas flow can be easily determined. A similar approach can be used to perform bifurcation analysis.
using the substrate feed concentration as the bifurcation parameter. In this case two media vessel can be used to feed the fermenter. The two vessels should have significant difference in their substrate concentration, probably one with a relatively high content while the other is devoid of substrate. Using high performance pumps, different levels of substrate feed concentration can be accomplished by varying the ratio of the feeds from the two media vessels such that the total flow rate to the reactor and hence the dilution rate is kept constant. The feed substrate concentration is simply the weighted average of the substrate concentration in the two vessels.

By all means, it is highly recommended to improve the current experimental measurements. The most troubling measurement is that of the dilution rate. Indeed this is the single most important measurement in many experiments, particularly those in which the dilution rate is investigated as a bifurcation parameter. Currently, an external scale is fixed to the wall of the fermenter and calibrated to read the volume of the liquid phase. The mass of the feed vessel is measured and collected in a timely manner using an external balance. The feed mass flow rate is determined as the rate of change of the feed vessel mass. The feed volumetric flow rate is directly calculated knowing the density of the feed media. The reported dilution rate is calculated as the ratio of the feed volumetric flow rate to the liquid phase volume in the fermenter. All of these measurements and averaging do not result in a sufficiently accurate determination of the dilution rate. Therefore, it is suggested to examine the possibility of finding an approach that will offer a direct and fast
measurement of the dilution rate. One possibility is to inject the reactor with an inert substance the concentration of which can be traced online. Assuming perfect mixing, which is a very reasonable assumptions for such small scale fermenters, the dilution rate can be easily determined from the rate of decay of the trace substance concentration. In addition, the inlet flow rate to the fermenter can be made more steady by replacing the pulsating pumps, currently in use, by high performance continuous pumps, which will reduce the naturally occuring system disturbances. Furthermore, the inlet air flowrate measurement can be improved by replacing the current measuring device by modern mass-flow meters. Finally, it is recommended to use more rigid tubings than the ones currently used in order to reduce the wearing of the tubes which continuously alters the inlet flow rate. This is particularly useful for long term experiments.

As part of the future mathematical modeling tasks, it is recommended to add some chemical structure to the media in both the age distribution model and the hybrid mass-age distribution model. The proposed PBE models would become more biologically acceptable if reasonable assumptions were made to augment to them ethanol as a by-product and/or supplementary substrate, as well as dissolved oxygen and exhaust carbon dioxide concentrations. Furthermore, this accessory makes it easier to compare model predictions to actual online signals and by far makes the models more useful for optimization and control purposes.
Bibliography


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Appendix: Unpublished Experimental Results

Many experiments have been performed towards achieving the goals of our current investigation of autonomously oscillating yeast cultures. However, the experimental results presented and discussed in the previous chapters represent only a subset of our experimental findings. The quality of the experimental results which were not discussed in the main body of this dissertation was compromised due to various factors. Some of the factors that have affected the quality of the obtained results are that the amount of data collected is not sufficient to decisively reach conclusions that are beyond reasonable doubt. Inaccuracies in the data collected is caused by variations in the operating conditions due to, e.g., problems in the pumps, controllers, agitator, and so on; or due to the appearance of a contaminant at a later stage of the experiment which requires a subjective judgment of when to draw the line between good results and bad results.

In the following sections, the results of three of the experiments that were not discussed in the previous chapters will be presented briefly. The quality of these results is not as good as that of results discussed thus far, therefore, only a few cautious conclusions can be drawn from them. Nevertheless, these cautious conclusions can still offer important insights towards the understanding of the autonomous oscil-
lations. This presentation is intended to stress the significance of these experiments and the importance of attempting to reproduce the results obtained.

**Materials and Methods**

The following materials and methods were utilized throughout the experiments discussed in this appendix. The microorganism used in this study was *Saccharomyces cerevisiae*, H1022 (ATCC 32167). The strain was kept on agar slants at 4°C. Inoculum was prepared by transferring a single colony from the slant to a flask containing 30 ml YEP (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 0.1 ml PPG 2000). The inoculum was grown in a shaker (Thermolyne) for 22 hours at 30°C and 180-rpm. The culture was inoculated on a 6% (v/v) basis.

Batch and continuous cultures were run in a Bioflo 3000 (New Brunswick) fermenter with a working volume of 1.0 L using medium D with increased concentrations of salts and vitamins [49]. It contained 30 g/L glucose, 9 g/L (NH₄)₂SO₄, 2.88 g/L (NH₄)₂HPO₄, 1.32 g/L KCl, 0.69 g/L MgSO₄·7H₂O, 0.42 g/L CaCl₂·2H₂O, 3.51 mg/L CuSO₄·5H₂O, 21.6 mg/L FeCl₃·6H₂O, 15.8 mg/L MnSO₄·H₂O, 13.5 mg/L ZnSO₄·7H₂O, 45 mg/L biotin, 90 mg/L m-inositol, 45 mg/L Ca-pantothenate, 9 mg/L thiamine, 2.25 mg/L pyridoxal-hydrochloride, 0.1 ml/L PPG 2000 and 0.7 ml/L sulfuric acid. Glucose, PPG and sulfuric acid with 80% water were autoclaved for 30 minutes at 121°C. The water used in the preparation of the glucose solution is pre-autoclaved for one hour before the addition of glucose, PPG and sulfuric acid. Vitamins, salts and the remaining water were filter sterilized using
0.22 μm Millipore filters. The water used in the preparation of the salt and vitamin solutions was autoclaved for 30 min and then cooled to room temperature prior to use. The temperature was maintained at 30°C and agitation rate was set to 800 rpm. The pH was kept at 5.0 using 4N NaOH. The culture medium was aerated with a flow of 1.5 SLPM. A DO probe (Ingold) and pH electrode (Ingold) measured dissolved oxygen and pH, respectively.

The signals from the Bioflo 3000 were collected through an A/D converter, monitored and recorded on a PC using Biocommand software (New Brunswick). The gas analyzer for online measurements of exhaust oxygen, carbon dioxide and ethanol was Industrial Emission Monitor Type 1311 (Brüel & Kjaer). The measurements were also collected through an A/D converter, and were monitored and recorded using the same software.

An external scale was fixed to the wall of the fermenter and calibrated to read the volume of the liquid phase. The mass of the feed vessel was measured and collected in a timely manner using an external balance. The feed mass flow rate was determined as the rate of change of the feed vessel mass. The feed volumetric flow rate was directly calculated knowing the density of the feed media. The reported dilution rate was calculated as the ratio of the feed volumetric flow rate to the liquid phase volume in the fermenter. Due to the slight changes in the feed flow rate over extended periods of operation and the difficulty in controlling the level of the liquid
phase, it was estimated that the absolute error in the reported dilution rates is in the order of ±0.005 hr⁻¹.

**Experiment 1**

The objective of this experiment was to attain a non-oscillatory state at dilution rate of 0.15 hr⁻¹ where oscillations have been frequently observed. In this experiment, the strategy was to start with a batch culture. At the stationary phase of the batch growth, the culture was switched to the continuous operation at $D = 0.10$ hr⁻¹. It was found in a previous experiment that this dilution rate does not support oscillations. After one day of operating at this point the dilution rate was to be increased linearly in 48 hours to the desired value of 0.15 hr⁻¹. It is expected that this change in the dilution rate is slow enough to keep the system at a quasi-steady state. The dynamics of the system were then observed and recorded after the new dilution rate was established. The system dynamics at the onset and during the continuous operation in this experiment are shown in figure A1 in terms of the evolved CO₂ signal (—). The figure also shows the value of the dilution rate (---) at the different stages of the experiment.

Towards the end of the dilution rate ramp a few small amplitude oscillations appeared. The oscillations seemed to be dying out after the third cycle. In this experiment, however, a precipitation of insoluble salts blocked the inlet of NaOH feed, which is used for pH control. This precipitation led to very poor pH control. At process time 101 hours a sharp decrease in the pH signal was observed.
Figure A1: System response in terms of the exhaust CO$_2$ signal (---) to variations in the dilution rate. A stationary phase batch culture was switched to continuous operation with an initial dilution rate (- -) of 0.10 hr$^{-1}$. The system was controlled at this operating point for 26.5 hr. A 48 hr ramp was then applied to increase the dilution rate to 0.15 hr$^{-1}$. The system was controlled at these operating conditions for two more days.

This decrease was proceeded by a peak in the CO$_2$ signal and an inverse peak in the dissolved oxygen signal (data not shown). To enhance pH control the NaOH maximum flowrate was increased by 33% of its nominal value between process time 102 and 105 hr. After correcting the pH level, the maximum NaOH flowrate was reduced back to its nominal value to avoid excessive controller action. During the next 10 hours, the pH control was inefficient, and in some instances the pH value dropped to 4.5 before enough controller action was performed to bring it back to its set point value of 5.0. At process time 115 hr, the maximum of the corrective NaOH flowrate was increased again and pH control was satisfactory for almost 10
hours. During this time the precipitation gradually increased until the controller fin­nally failed and strong disturbances in the measured pH signal were observed again. Figure A2 shows the detailed system dynamics during the continuous operation at $D = 0.15 \text{ hr}^{-1}$ just after the end of the ramp and until the end of the experiment in terms of the exhaust CO$_2$ signal. The figure also shows the pH value. It is apparent that the system did not reach to an oscillatory state. However, it is not obvious whether the non-oscillatory state observed was because the system was kept at a quasi-steady state during the ramp and finally reached a stable steady state at the final dilution rate or that the oscillations were not sustainable due to the poor pH control and the associated disturbances. It is apparent from figure A2 that there is a clear correspondence between the evolved CO$_2$ signal and the noise in the pH signal. This was also true for the rest of the measured system variables such as the dissolved oxygen.

The tip of the NaOH feed inlet is positioned above the level of the liquid in the fermenter, however, due to the agitation some droplets of the fermenter medium reach the inlet and react with the incoming base to form the observed precipitation of insoluble salts. In order to overcome this problem in future experiments, it was recommended to operate the fermenter at lower working volumes and also to use less concentrated NaOH solution. It was found that these two precautions have been sufficient to completely eliminate the precipitation problem.
Figure A2: The failure of the pH controller due to the formation of salt precipitates at the inlet of the NaOH feed resulted in fluctuation in the fermenter pH value (- -) and consequently affected various system variables such as the exhaust CO$_2$ signal (—). It is not obvious if the oscillations at these operating conditions were suppressed due to the attainment of a stable steady state at the end of the dilution rate ramp or due to the continuous disturbances of the fermenter pH value.

**Experiment 2**

In this experiment, a non-oscillating continuous culture at $D = 0.155$ hr$^{-1}$ was switched to the batch mode. About 90% of the fermenter content was disposed and replaced with fresh media, then regular batch growth was permitted. At the stationary phase of the batch, the culture was switched to the continuous mode with a dilution rate of 0.145 hr$^{-1}$. Shortly after the switch ethanol was produced as evidenced by the increase in the hydrocarbon content of the exit gaseous stream. The produced ethanol was consumed within the first four hours of the continuous operation. Consistent with previous experimental data and with observations re-
ported in literature, the oscillations appeared only upon depletion of ethanol. The oscillations at this dilution rate, however, were not stable. Both the amplitude and the period of oscillations decreased over a period of 35 hours after the appearance of the first cycle. Then both the amplitude and the period slightly increased again during the next 20 hours before they eventually diminish and completely disappear. Figure A3 shows the evolved CO$_2$ signal as well as the ethanol concentration upon the switch of the batch culture to the continuous mode and during the next 85 hours of continuous operation. The ethanol concentration was estimated based on the value of the hydrocarbon content in the exit gas stream utilizing a calibration curve obtained in a previous experiment. The vessel containing the feed media was not replaced throughout the continuous growth; therefore, the substrate feed concentrations were constants during the continuous operation. According to the level sensor and balance measurements there was no indication that the operating volume of the fermenter or the dilution rate had changed during the continuous operation. Observing samples from the fermenter outlet stream under the microscope at different times of the continuous growth did not conclusively confirm the existence of contamination. Therefore, it is reasonable to assume that these specific continuous operating conditions do not support oscillations and hence it was not possible to sustain the oscillations.

It should be mentioned though that oscillatory dynamics have been observed at dilution rates in the vicinity of 0.145 hr$^{-1}$ and using medium D as the feed me-
Figure A3: At certain continuous operating conditions, the oscillatory dynamics may take several days to die out opposite to the common notion that these oscillations always appear or disappear spontaneously. The evolved CO$_2$ signal (—) and ethanol concentration (—- -) are shown upon the switch of a batch culture to the continuous operation at $D = 0.145$ hr$^{-1}$ and during the next 85 hours.

However, the results from this experiment along with the results from the next experiment confirm that at these dilution rates the system is very sensitive to the concentrations of substrates in the feed media. The composition of and the preparation procedure for medium D was discussed above in the materials and methods section. There is a number of factors that are difficult to control in the preparation procedure for medium D, resulting in some variation between the different preparations of the medium. The most important factor that cannot be controlled is the amount of water evaporated during the sterilization process in the autoclave. Although the variations between different preparations of medium D are small, these
variations could play a critical role in the appearance or disappearance of the oscillations at certain dilution rates. Regardless of the reason why the oscillations disappeared, this experiment reveal a very important feature of the autonomous oscillations of yeast cultures that has not been reported in literature. Typically, these oscillations has been regarded as spontaneously occurring and spontaneously disappearing. In this experiment, it is shown that under some circumstances the oscillations may not disappear spontaneously, but instead take several hours or even days to disappear. Population balance models, unlike metabolic models, do predict a similar behavior if a highly synchronized culture was subjected to operating conditions that do not permit sustained oscillations. In these cases, PBE models predict that unstable oscillatory dynamics prevail for some time before the steady state is eventually reached.

**Experiment 3**

In this experiment, another interesting feature of the autonomous oscillations of yeast cultures was observed. The oscillations not only possess the ability to prevail for considerable durations of time before they slowly and progressively disappear, but also in some situations the oscillations can slowly and progressively develop. Thus it was concluded, in opposition to the common understanding, that the autonomous oscillations in budding yeast do not always appear in a spontaneous fashion.
The objective of this experiment was to study the hysteresis phenomena in oscillating yeast cultures. More specifically, it was desired to determine the value of dilution rate at which the oscillatory dynamics disappear and the value at which they reappear. This experiment can be divided into two phases. In the first phase of the experiment, a stationary batch culture was switched to the continuous operation at $D = 0.158 \text{ hr}^{-1}$. The oscillations appeared spontaneously and were sustained for almost 54 hours. The dilution rate was then linearly reduced to $0.144 \text{ hr}^{-1}$ in 12 hours. The oscillations did not totally disappear, and some oscillatory components could still be determined through FFT analysis. Nevertheless, the system dynamics became less regular. This operating point was maintained for 30 hours, before a positive ramp was applied on the dilution rate to increase its value to $0.152 \text{ hr}^{-1}$ in 6 hours. The system dynamics became less regular and the oscillations disappeared as evidenced by the FFT analysis of the exhaust CO$_2$ signal. Data was collected at the new dilution rate for 25 hours. A problem was encountered with the data acquisition software, and there was no data collections for the next 10 hours. Figure A4 shows the system dynamics during the continuous operation of the first phase of experiment 3 in terms of the exhaust CO$_2$ signal (—). The variation in the dilution rate is also shown in the figure (— -).

In the second phase of this experiment, the data acquisition problem was fixed and a new session of the software used for data collecting was started. One hour after the start of the new session, the feed media vessel was replaced. Small ampli-
Figure A4: The effect of dilution rate (---) variation on the system dynamics in the first phase of experiment 3. The autonomous oscillations at \( D = 0.158 \text{ hr}^{-1} \) in terms of the evolved \( \text{CO}_2 \) signal (—) were quenched by reducing the dilution rate linearly to 0.144 \( \text{hr}^{-1} \). The oscillations were not retrieved upon the linear increase of the dilution rate to 0.152 \( \text{hr}^{-1} \) in 6 hours.

At the time the oscillation started to appear upon the switch of the feed media vessel. These oscillations slowly and progressively increased in amplitude and period until regular oscillatory pattern completely developed in almost 35 hours. The dilution rate had slightly decreased to 0.151 \( \text{hr}^{-1} \) after the replacement of the feed media vessel. This operating point was maintained for 40 hours before decreasing the dilution rate linearly to 0.143 \( \text{hr}^{-1} \) in 6 hours. A new phenomenon has been observed after performing the negative ramp in dilution rate. The oscillatory dynamics became less regular for almost 45 hours in the sense that only 4 isolated peaks in the \( \text{CO}_2 \) signal were observed. Afterwards, the typically observed regular oscillations autonomously
appeared without noticeable changes in the operating conditions. Figure A5 shows the system dynamics during the continuous operation of the second phase of experiment 3 in terms of the exhaust CO$_2$ signal (—). Figure A5 also shows the variation in the dilution rate (—). Note that the data shown in figure A5 is not an immediate continuation of the data presented in figure A4 because there was a 10 hour gap in data collection as mentioned earlier.

Figure A5: The effect of dilution rate (—) variation on the system dynamics in the second phase of experiment 3. Regular oscillatory pattern slowly and progressively developed after the replacement of the feed media vessel at $D = 0.151$ hr$^{-1}$ in terms of the evolved CO$_2$ signal (—). The dilution rate was linearly decreased to $0.143$ hr$^{-1}$ in 6 hours. After long transients, which lasted for almost 45 hours the oscillatory pattern was re-established.

As discussed in the previous section, the new media vessel used one hour after the start of the second phase in experiment 3 might have slightly different substrate concentrations. This presumed variation in the substrate feed concentrations could
have modified the environmental condition such that the oscillations would appear. Again the slow appearance of the oscillations has not been reported in literature.

This phenomenon cannot be predicted using structured, metabolic models such as the cybernetic model by Jones and Kompala (1999) [21]. However, PBE models do predict such behavior in certain situations. For example, the hybrid mass-age distribution model (see chapter 4) predicts that if a continuous culture was at a steady state then the dilution rate was changed slowly to a value that support oscillatory dynamics, the oscillations will not appear spontaneously, but will slowly and progressively develop.
**Vita**

Abdelqader M. Zamamiri, is a doctoral student in the Department of Chemical Engineering, at Louisiana State University. He received his master of science degree in chemical engineering from Louisiana State University, Baton Rouge, Louisiana, at the commencement of Fall 1998. His thesis was entitled “Analysis of Oscillating Yeast Cultures”. He received his bachelor's degree in chemical engineering from the University of Jordan, Amman, Jordan, at the commencement of Spring 1994. He ranked first out of 69 classmates.

In Summer 1993, and during his undergraduate study, he spent three months of practical training in the Research and Development Department of De Danske Sukker Fabrikker, Driftkisk Laboratorium, Denmark. He worked in a research project focusing on the drying of sugars. Also, during the last semester of his undergraduate study he worked as a research assistant. Before pursuing his graduate study, the author worked as a teaching and research assistant in the Department of Chemical and Petroleum Engineering at United Arab Emirates University, Al-Ain, United Arab Emirates, for two years, 1994-1996. He assisted in teaching more than ten distinct courses and labs in all levels of undergraduate chemical engineering. He also worked in research projects in the area of seawater desalination.

While at Louisiana State University, he designed and performed coordinated sets of experiments to study autonomously oscillating yeast cultures. Furthermore,
he developed, solved and analyzed population balance models of budding yeast. Zamamiri, also assisted in writing a proposal to the National Science Foundation and co-authored and reviewed a number of scientific journal papers. He received a number of awards and assistantships including the Graduate School Supplement Award, Tuition Waiver Award and Graduate Assistantship. The author's current area of research is mathematical modeling of biochemical systems. His research interests also include mathematical modeling and simulation of chemical systems, optimization and control.

Zamamiri will receive the degree of doctor of philosophy in chemical engineering at the commencement of Spring 2001.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Abdelqader M. Zamamiri

Major Field: Chemical Engineering

Title of Dissertation: Analysis and Mathematical Modeling of Autonomously Oscillating Yeast Cultures

Date of Examination: December 13, 2000

Approved:

[Signatures of Major Professor and Chairman, Dean of the Graduate School, and Examining Committee]

Date of Examination: December 13, 2000

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.